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## EXPERIMENTAL STUDIES ON THE DURATION OF LIFE. XI. DENSITY OF POPULATION AND LIFE DURATION IN *DROSOPHILA*<sup>1</sup>

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### *Introduction*

IN an earlier paper in this series Pearl and Parker (49) showed, by the compilation of then existing statistics, that in wild, sepia and quintuple stocks of *Drosophila* there was a definite relationship between density of population in the experimental bottles and the duration of life of the flies. Owing to the meagerness of the material and its limitations in respect of density range, it was not possible from the purely statistical data to determine with any accuracy the form of the regression of duration of life upon density. We pointed out that *ad hoc* experimentation would be necessary to get a satisfactory account of this and other interesting points adumbrated in the preliminary statistical study. In the five years which have elapsed since the publication of the paper referred to there has been carried out in our laboratory a great deal of experimental work on the density problem, which will be reported in this and subsequent papers. Only a brief preliminary paper (74) has hitherto been published on this experimental work on the influence of density of population on life duration.

In our first study of the matter (49) it was apparent that in the case of the wild type long-lived flies the re-

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gression of duration of life upon density of population was non-linear. The form of the curve suggested that the relationship between these two variables was of such sort that the lowest densities were not the optimal for life duration. On the contrary, it appeared that the curve of mean duration of life gradually rose from the lowest densities to a maximum in the region of initial densities of 35 to 40, and after that point fell off with increasing initial density. Two defects in the material, however, prevented the drawing of any certain conclusions on these points. In the first place it was necessary to group densities fairly heavily, and in the second place the highest initial density in the material was only a little more than 90 flies per bottle.

Our first series of experiments were designed primarily to remedy these two defects in the earlier statistical material. To this end 20 different densities were used, closely spaced at the earlier end of the curve. The highest density was 200 flies per one ounce vial. It was thought that the experimental points were so chosen as to be bound to give a clear and accurate picture of the major portion, at least, of the whole density-duration of life curve. As a matter of fact this proved to be the case, except for the extreme upper end of the curve.

#### *Material and Methods*

The first series of experiments reported upon in this paper were carried out in general accordance with the technique described in (27) and subsequent papers in this series. The food used was the standard banana-agar medium described in (27) and also in (78). The experiments were all carried out in one ounce, screw top vials, stoppered with cotton plugs. These vials were placed in an electric incubator running at 25° C. The bottles were examined daily, and the dead flies removed and their age at death recorded, to the degree of accuracy implied in examination at 24 hourly intervals. The living flies were at the same time transferred to fresh bottles of newly prepared food.

TABLE 1  
PLAN OF EXPERIMENTS

Number of flies in each bottle at start. Initial density	Number of bottles at each indicated initial density	Total number of flies in experiments at start
2	150	300
4	80	320
6	50	300
8	40	320
10	30	300
12	30	360
15	20	300
20	10	200
25	10	250
35	10	350
45	10	450
55	10	550
65	10	650
75	10	750
85	10	850
95	10	950
105	10	1050
125	10	1250
150	10	1500
200	10	2000
Totals	530	13,000

In each bottle was put at the start an equal number of male and female flies, except that when the initial density was an odd number, one half of the bottles of that density had one more female than there were males, while the other half of the bottles in that series had each one more male than there were females. There were then in the whole lot at the start 6,500 males and 6,500 females.

Since the size of all the bottles was the same (1 oz.) and the amount of banana-agar put in each bottle was always the same, density of population may most simply be indicated as the number of flies per bottle, and this usage will be followed throughout the paper.

The flies used were all wild type *Drosophila melanogaster* of our Line 107 (see (32) for history of this line).

The mathematical analysis of the experimental records has followed actuarial lines. In the tables in this paper the following kinds of quantitative information, which

need some special explanation, are tabulated and discussed in the text.

*Survivorship distributions* (symbol  $l'_x$ ). These give the number of individuals surviving at the beginning of each indicated age period, on the basis of 1,000 flies starting together at emergence. The absolute numbers involved are given in each case. The  $l'_x$  values are ungraduated and unsmoothed.

*Death rates* (symbol  $q'_x$ ). These are the observed death values, unsmoothed, got by dividing the observed deaths in a given age period by the number living at the beginning of that period, or in some cases by the number of fly-days exposed to risk. In the first case the mortality rates are per thousand flies living at the time. In the second case they are rates per thousand fly-days exposed to risk of death.

*Mean density per bottle* (symbol *M.D.*). The density of population, as defined above, obviously must change with the history of the bottle. For example, suppose a bottle started with 2 flies. At the end of say 14 days one of these flies dies. Up to that time the density of population in the bottle has been 2. After that time, and on till the death of the lone survivor, the density is 1. Now the first fly lived its entire life of 14 days exposed to a density of 2. But the second fly lived 14 days of its life exposed to a population density of 2, and, let us say, the remaining 18 days of its life exposed to a density of 1. So then the mean density to which it was exposed over its entire life of 32 days was:

$$\begin{array}{r} 14 \times 2 = 28 \\ 18 \times 1 = 18 \\ \hline 32 \quad 46 \end{array}$$

$$\text{Therefore, the mean density} = \frac{46}{32} = 1.44$$

In this way the mean density to which the fly was exposed during its whole life time was calculated for each of the 13,000 flies in the experiment. It was a long and laborious bit of computation.



Weighted means were then made of the mean densities for all flies starting at the same initial densities. The basis of the weighting was the frequency of occurrence.

Naturally in so extensive and laborious a piece of experimental work there were accidents and errors due to the frailty of human eyes, hands, and brains. Some flies were accidentally lost in transfers, some records probably were set down in the wrong column, etc. But careful examination of the records shows the number of such experimental errors was not great enough to affect sensibly any of the essential results of the study. In the computations the assumption was made that any fly lost at an unknown date was lost at the mid-point of the history of the bottle from which it came. On the theory of probability, and in the absence of definite information, it can be argued that this is the most probable time for the loss to have occurred.

#### *Survivorship Curves*

We may begin the examination of the results with the  $l_x$ , or survivorship distributions. These are given in Table 2. In this table I. D. means Initial Density, that is, the number of flies per bottle at the start, and A. M. D. means Average Mean Density to which each fly was exposed in its entire life time.

It is at once evident from these distributions, and from the graphical representations of them shown in Figs. 1 and 2, that density of population has a marked influence upon life duration.

The highest line in each diagram is that for initial density 35. This means that at about that density the flies, on the whole, survived longest. Much lower or higher initial densities were less favorable to survival under the conditions of the experiments. The form of the survivorship distribution undergoes a gradual alteration on either side of the optimal density. This is particularly true as we go towards higher densities, until finally at density 200 the survivorship curve on arithlog





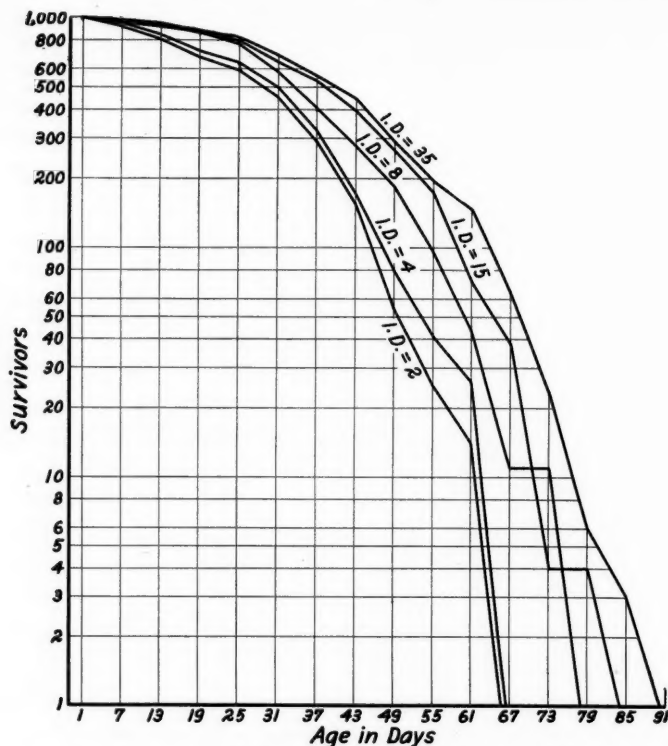


FIG. 1. Survivorship distribution for initial densities of 2, 4, 8, 15, and 35 flies per one ounce bottle. Data from Table 2.

paper approaches the straight diagonal line which indicates a constant death rate at ages.

#### *Age Specific Death Rates*

It is desirable next to examine in detail the death rates at ages, for the different densities. The data are exhibited in Table 3, which also shows the mean density of population to which the flies dying at each age period had been subjected up to the time of death.

A study of Table 3 shows that the specific death rates in these experiments conformed in their behavior to the following principles, with, of course, fluctuations due to sampling errors:

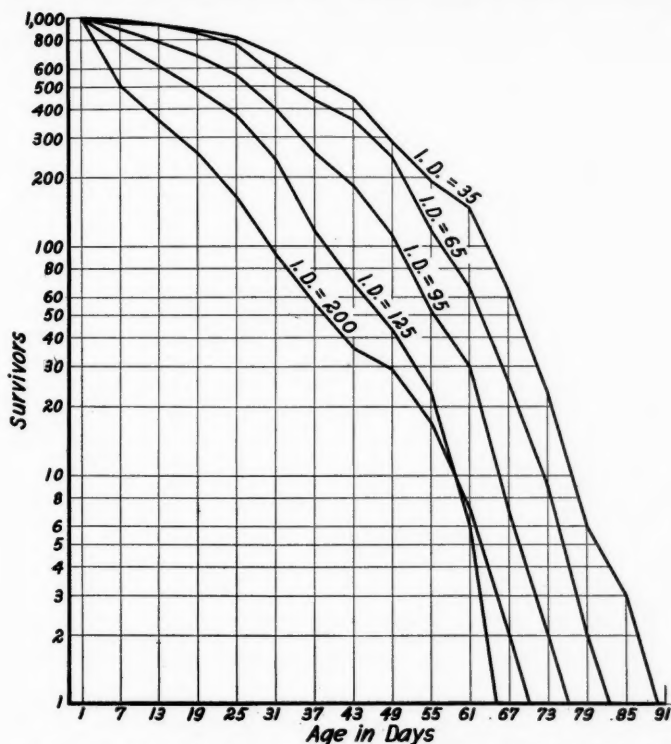


FIG. 2. Survivorship distribution for initial densities of 35, 65, 95, 125, and 200 flies per one ounce bottle. Data from Table 2.

1. The death rates, at all densities up to about 100 flies per bottle, tend to increase with age, in the normal manner expected from *Drosophila* life tables (66), and from all our previous studies on the mortality of this form. At densities beyond 100 to 125 flies per bottle there is a high death rate at the start (lowest ages), which tends to fall with advancing age up to roughly the middle of life, and thereafter to increase with advancing age.

2. At all ages the death rates start at a relatively high point in the lowest density (2 flies per bottle) and tend thereafter to *decline* with increasing density until den-

### TABLE 3

#### AGE SPECIFIC DEATH RATES

Age in days	I. D. = 2		I. D. = 4		I. D. = 6		I. D. = 8		I. D. = 10	
	M. D. 1000		M. D. 1000		M. D. 1000		M. D. 1000		M. D. 1000	
	$q'_x$		$q'_x$		$q'_x$		$q'_x$		$q'_x$	
1-6 .....	1.97	82	4.00	49	6.00	46	7.96	50	10.00	55
7-12 .....	1.89	125	3.89	118	5.77	40	8.00	37	9.87	58
13-18 .....	1.92	165	3.66	156	5.60	84	7.39	58	9.33	75
19-24 .....	1.72	118	3.35	101	5.57	119	7.36	112	9.07	86
25-30 .....	1.79	236	3.49	218	5.32	176	7.26	242	9.01	120
31-36 .....	1.75	357	3.11	353	5.13	239	6.49	301	8.49	178
37-42 .....	1.71	469	3.02	465	4.83	322	6.25	316	7.75	324
43-48 .....	1.59	651	2.86	543	4.47	439	6.05	333	7.33	319
49-54 .....	1.45	533	2.57	476	4.31	435	6.11	481	6.97	391
55-60 .....	1.88	429	2.54	364	4.29	500	5.56	556	6.84	487
61-66 .....	1.28	1000	2.56	1000	4.00	846	5.40	750	7.09	900
67-72 .....	.....	.....	.....	.....	3.59	500	.....	.....	7.58	500
73-78 .....	.....	.....	.....	.....	.....	.....	4.50	1000	.....	.....
79-84 .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
85-90 .....	.....	.....	.....	.....	3.91	1000	.....	.....	6.19	1000

[illegible]



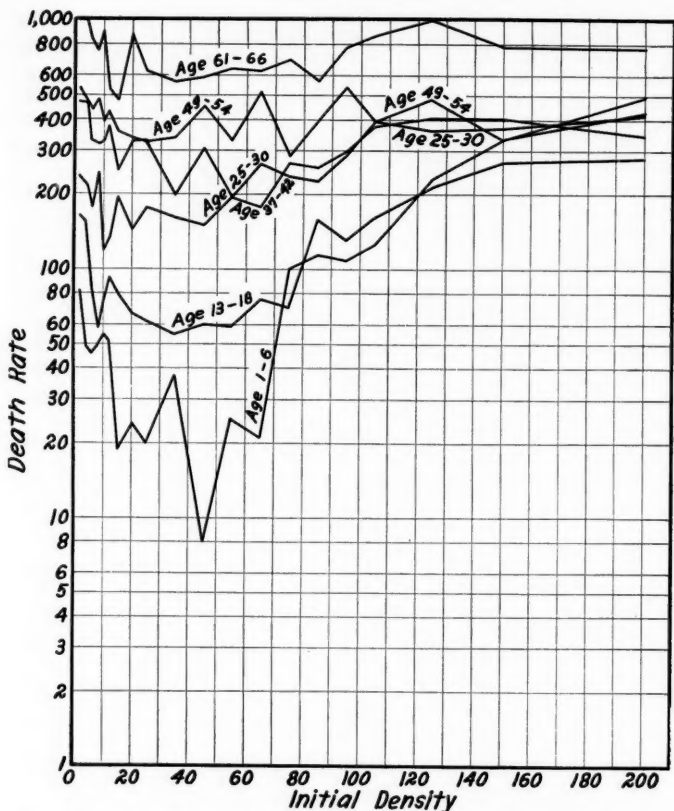


FIG. 3. Changes in specific death rates with advancing initial densities of population, for six age groups.

sities of roughly 35 to 55 flies per bottle are reached. Thereafter, as density of population increases, the course taken by the specific death rates is different in the different age groups. In the youngest age groups the death rates increase steadily with increasing density, after the optimal density is passed, right away to the highest densities tried in these experiments. But from the age group 25-30 on to the end of the life span there is virtually no increase in death rates associated with advancing densities of population, after a density of 100 flies per bottle



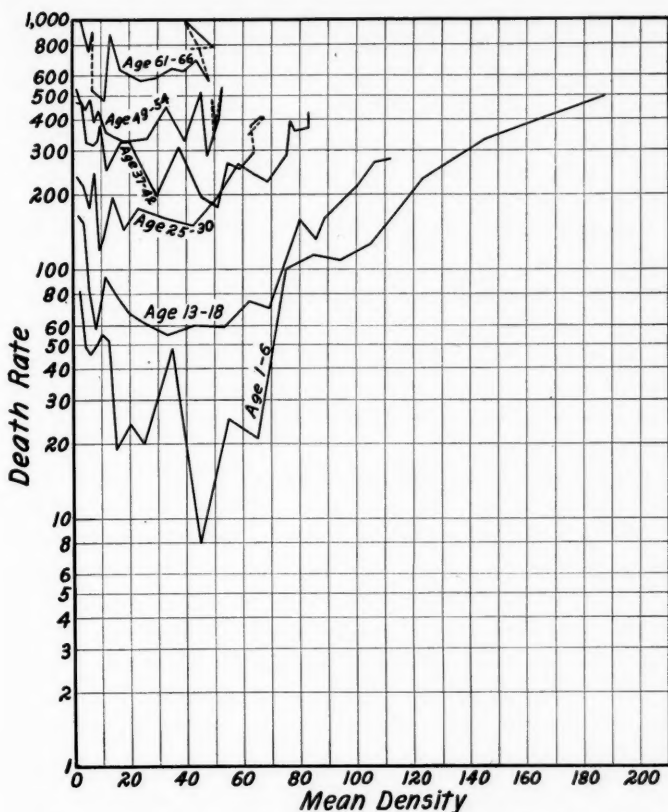


FIG. 4. Changes in specific death rates with advancing mean densities of population, for six age groups.

is reached. In general, as is plainly shown in Figs. 3 and 4, the death rate curves, for constant age and changing density, tend to become flatter (that is to approach more nearly to straight horizontal lines) as age increases.

These relations are shown graphically in Figs. 3 and 4. In both of these diagrams density is plotted as abscissa, and specific death rate as ordinate. In Fig. 3 initial densities are used, and in Fig. 4 mean densities to which the flies dying had been subjected. The specific death rates for six age groups only are plotted, in order not to crowd

the figures with too many lines. The age groups chosen for plotting are the following: 1-6, 13-18, 25-30, 37-42, 49-54 and 61-66. It will be noted in Fig. 4 that in the three higher age groups the lines proceed occasionally in a negative abscissal direction, especially at the highest mean densities. This "back-tracking" of the lines is indicated by dots.

The facts which have been set forth regarding the influence of population density upon mortality seem to support the following conclusions:

(1) The rate of mortality of *Drosophila* is profoundly influenced by density of population, that is, by the number of flies together occupying a limited universe in which volume of air, volume of food, and area of food surface are constant.

(2) There is an optimal density of population for *Drosophila*, under the conditions of these experiments. This optimal density of population falls somewhere in the region of 35-55 flies per one ounce bottle containing 8 c.c. of food substrate. At densities of population above and below the optimum the specific death rates are higher, at all ages, than they are at the optimum.

(3) The deleterious effect of non-optimal densities of population is most pronounced at the beginning of imaginal life. In the first few days after the fly emerges from the pupa case it is extremely sensitive to influences consequent upon the size of the group of which it is one component member. As the fly grows older differences in either initial or mean density of population have associated with them relatively smaller differences in rate of mortality.

#### *Biometric Constants*

The nature of the changes in duration of life associated with different densities of population may be shown in a different way, by calculating the ordinary biometric constants from the distribution of deaths in respect to age. These constants are exhibited in Table 4.

The mean values for duration of life given in Table 4 are shown graphically in Fig. 5.

TABLE 4  
BIOMETRIC CONSTANTS FOR DURATION OF LIFE

Initial density	Weighted average mean density to which flies had been subjected up to time of death	Duration of life in days		
		Mean	Standard deviation	Coefficient of variation
2	1.77	27.31 $\pm$ .58	14.47 $\pm$ .41	52.9 $\pm$ 1.9
4	3.30	29.32 $\pm$ .60	14.51 $\pm$ .42	49.8 $\pm$ 1.8
6	5.00	34.45 $\pm$ .65	15.46 $\pm$ .46	44.7 $\pm$ 1.6
8	6.68	34.20 $\pm$ .61	15.10 $\pm$ .43	44.1 $\pm$ 1.5
10	8.15	36.22 $\pm$ .72	16.93 $\pm$ .51	46.7 $\pm$ 1.7
12	9.72	34.31 $\pm$ .61	16.54 $\pm$ .43	48.2 $\pm$ 1.5
15	12.42	37.92 $\pm$ .66	15.96 $\pm$ .47	42.1 $\pm$ 1.4
20	16.69	37.07 $\pm$ .55	14.72 $\pm$ .39	39.7 $\pm$ 1.2
25	20.68	37.47 $\pm$ .49	15.39 $\pm$ .35	41.1 $\pm$ 1.1
35	28.85	39.43 $\pm$ .67	17.55 $\pm$ .47	44.5 $\pm$ 1.4
45	37.23	37.46 $\pm$ .51	15.19 $\pm$ .36	40.5 $\pm$ 1.1
55	44.65	40.04 $\pm$ .53	17.14 $\pm$ .37	42.8 $\pm$ 1.1
65	53.16	35.25 $\pm$ .45	15.97 $\pm$ .32	45.3 $\pm$ 1.1
75	59.66	32.34 $\pm$ .46	17.93 $\pm$ .32	55.4 $\pm$ 1.3
85	66.95	30.10 $\pm$ .36	15.06 $\pm$ .26	50.0 $\pm$ 1.0
95	74.50	27.17 $\pm$ .36	15.95 $\pm$ .25	58.7 $\pm$ 1.2
105	80.36	24.20 $\pm$ .32	14.87 $\pm$ .23	61.5 $\pm$ 1.2
125	94.38	19.60 $\pm$ .28	14.31 $\pm$ .20	73.0 $\pm$ 1.4
150	111.88	16.17 $\pm$ .24	13.75 $\pm$ .17	85.0 $\pm$ 1.7
200	144.47	11.93 $\pm$ .20	12.85 $\pm$ .14	107.7 $\pm$ 2.1

Between initial densities of 2 and 15 flies per one ounce bottle the mean duration of life increases rapidly with increased density. Between densities of 15 and 55 flies per bottle there is a slow and gradual increase in mean duration of life. In fact it is not certain that this region of the curve does not really represent a plateau of optimal density, in which region small differences in density make no significant difference in mean duration of life. After a density of 55 flies per bottle is passed the mean duration of life declines steadily with advancing density.

The form of the upper limb of the curve of decreasing mean duration of life with increasing density of population suggests that the curve is tending to approach a constant level or asymptote at extremely high densities.

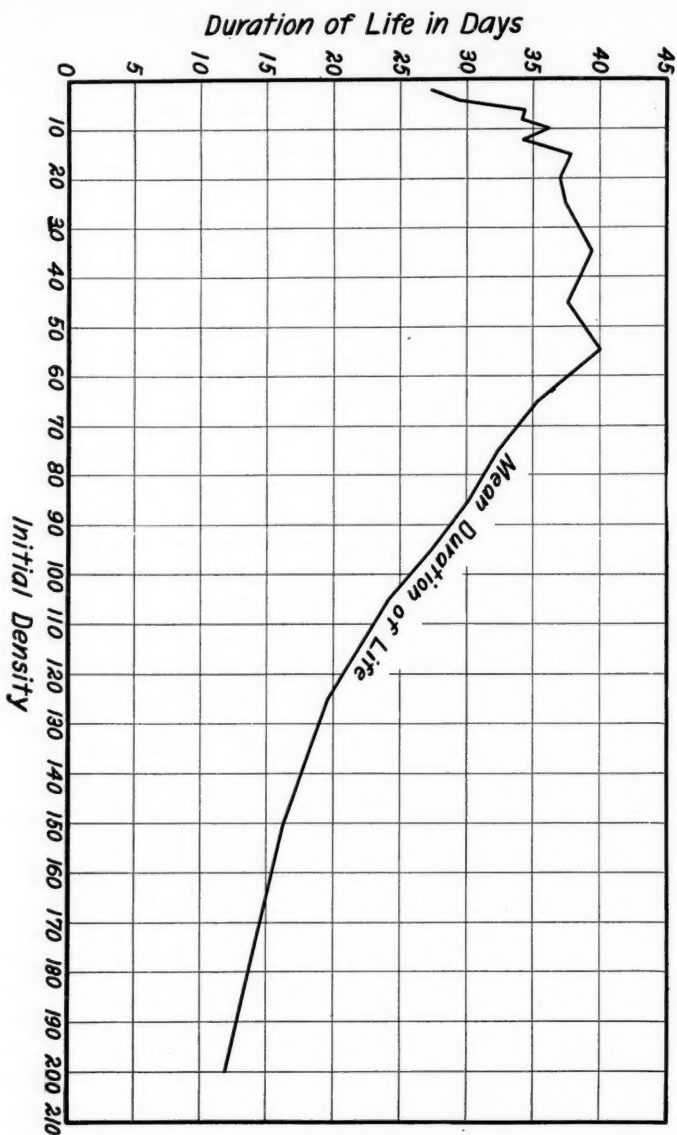


FIG. 5. Mean duration of life of wild type *Drosophila* at different densities of population.

To test this point a further series of experiments was undertaken, with the results shown in Table 5 and Fig. 6. The general technique of these experiments was identical with that which has already been described earlier in this paper. The flies used were wild type, Line 107.

TABLE 5  
BIOMETRIC CONSTANTS FOR DURATION OF LIFE  
*Repetition of Experiments with High Densities*

Density. Flies per one ounce bottle	Duration of life in days		
	Mean	Standard deviation	Coefficient of variation
5	35.98 $\pm$ .51	15.94 $\pm$ .36	44.29 $\pm$ 1.19
25	41.91 $\pm$ .44	17.11 $\pm$ .31	40.83 $\pm$ .86
50	41.60 $\pm$ .51	16.22 $\pm$ .36	39.00 $\pm$ .99
75	37.01 $\pm$ .47	16.50 $\pm$ .34	44.58 $\pm$ 1.21
100	33.07 $\pm$ .45	15.56 $\pm$ .32	47.07 $\pm$ 1.15
200	17.99 $\pm$ .49	10.65 $\pm$ .34	59.20 $\pm$ 1.61
300	13.79 $\pm$ .24	10.39 $\pm$ .17	75.32 $\pm$ 1.80
400	12.60 $\pm$ .19	9.44 $\pm$ .14	74.90 $\pm$ 1.56
500	10.98 $\pm$ .16	9.19 $\pm$ .12	83.66 $\pm$ 1.95

Allowing for the difference in the scale of plotting the abscissae, it is evident that the experiments leading to Table 5 and Fig. 6 fully confirm the results exhibited in Table 4 and Fig. 5. Furthermore Table 5 and Fig. 6

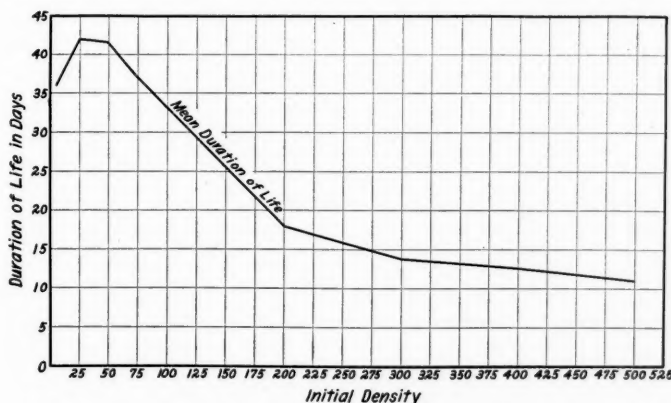


FIG. 6. Mean duration of life of wild type *Drosophila* at different densities of population. Data from Table 5.

show clearly the very gradual decline of mean duration of life with increasing densities of population after a density of 200 flies per one ounce bottle has been passed. Beyond this density little further effect on duration is produced by greater crowding.

An examination of the standard deviations in Tables 4 and 5 indicates that variation in duration of life is substantially homoscedastic relative to changing density of population, certainly at all except possibly extremely high densities.

The survivorship distributions at the extremely high densities approximate closely to the "straight diagonal line" type of life curve which has been discussed in an earlier paper in this series (66). This is shown in Fig. 7.

#### *New Synthetic Food and Density*

The results so far described regarding the influence of density of population upon duration of life have been many times confirmed during the past five years in this laboratory, by experiments conducted under a variety of conditions relative to other things than density of population. But always we get the same characteristic curve of mean duration of life shown in Figs. 5 and 6. Most of these experiments, however, have been carried out with the old banana-agar food medium.

Inasmuch as the general ecological conditions are somewhat different in the bottles with the new synthetic food S-101 (79), which we now use in all our *Drosophila* work, from what they are with the old banana-agar medium, it seemed desirable to carry out density experiments with this new food medium. This we have done, with the assistance of Mrs. Florence Barclay White and Miss Sophia Gould.

The plan of the experiments was essentially the same as that of the earlier work described in detail above, except that the new synthetic food medium was used in place of banana-agar. It was made and seeded with yeast according to the method described in (79). One ounce bottles were used in the earlier work, so that the

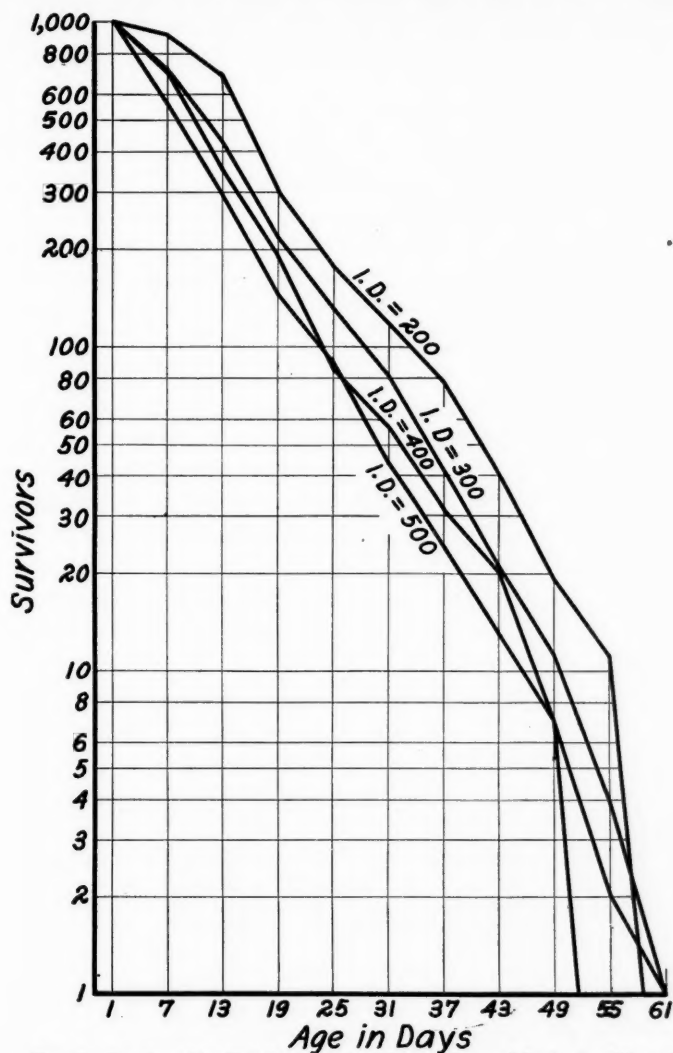


FIG. 7. Survivorship distribution of wild type *Drosophila* in densities of 200, 300, 400, and 500 flies per one ounce bottle. The abscissae are absolute ages in days.

densities are strictly comparable. The flies used were our wild line 107. The bottles were kept in an incubator at 25° Centigrade.

The life table death rates found in the experiment, for all ages taken together, are shown in Table 6. The total number of flies in the experiment was 2,081. The  $q'_x$  values tabled are computed on the basis of the number of fly-days exposed to risk. The data as to deaths were observed daily, but were grouped into 5-day age groups for computation. The exposure to risk for each 5-day period was determined as follows:

Age	Number exposed at beginning of each day
$x$	$l_x$
$x+1$	$l_x - 1/5 d_x$
$x+2$	$l_x - 2/5 d_x$
$x+3$	$l_x - 3/5 d_x$
$x+4$	$l_x - 4/5 d_x$
Total	$5 l_x - 2 d_x$

Table 6 also gives the biometric constants for the duration of life in these experiments.

TABLE 6

LIFE TABLE DEATH RATES AND BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF *DROSOPHILA MELANOGASTER* ON SYNTHETIC FOOD, AT DIFFERENT DENSITIES OF POPULATION

Initial density. Flies per one ounce bottle	Death rate per 1,000 fly-days exposed	Duration of life in days		
		Mean	Standard deviation	Coefficient of variation
2	30.04 ± 1.82	32.79 ± 0.73	11.89 ± 0.52	36.3 ± 1.8
4	31.05 ± 1.88	31.71 ± .86	14.03 ± .61	44.2 ± 2.3
8	30.26 ± 1.59	32.50 ± .75	14.08 ± .53	43.3 ± 1.9
16	30.10 ± 1.58	32.72 ± .67	12.62 ± .48	38.6 ± 1.7
32	27.44 ± 1.32	35.94 ± .65	13.45 ± .46	37.4 ± 1.5
64	31.05 ± 1.49	31.71 ± .62	12.61 ± .44	39.8 ± 1.6
128	37.61 ± 1.27	26.09 ± .55	15.94 ± .39	61.1 ± 2.0
256	74.36 ± 1.75	12.95 ± .35	14.11 ± .24	109.0 ± 3.5

The mean durations of life of Table 6 are shown graphically in Fig. 8.



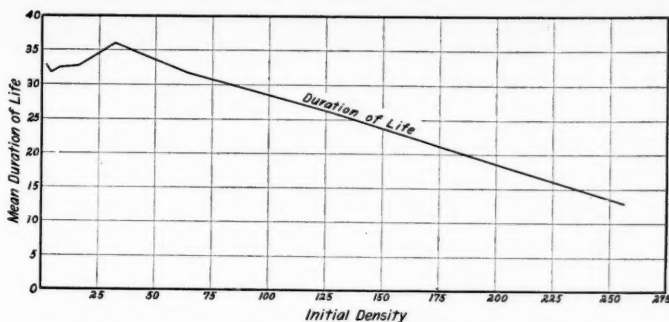


FIG. 8. Mean duration of life of *Drosophila* at eight different densities of population on synthetic food, S-101.

The results of these experiments confirm those of the earlier work with banana-agar food. The optimum density of population with respect to duration of life is at 32 flies per bottle in these experiments. The general trend of the mean duration of life curve is essentially the same as that of the corresponding curves presented earlier in this paper.

#### *Changing Density*

With the results in hand which have been described, various experiments looking to the further analysis of the effect of density upon duration of life suggest themselves. Here we shall report only one such series, leaving for future discussion other angles of the problem.

It has been seen that a density of 35 flies per one ounce bottle is at least near the optimal density for duration of life. On the other hand a density of 200 flies is extremely deleterious to duration of life. Suppose now that we set up a series of bottles according to the following plan.

A. Bottles at initial density 35, serving as controls, and not further manipulated except that on the morning of the 16th day of their age the then surviving flies in each bottle were etherized and the tips of the wings of half of them were clipped off with iridectomy scissors, and then they were returned to their proper bottles.

B. Bottles at initial density 200, serving as controls and not further manipulated, except that on the morning of the 16th day of their age they were etherized and counted.

C. Bottles at initial density 200, in which the mortality is allowed to go on at the normal rate for this density for 15 days. On the morning of the 16th day the number of flies in each such bottle is brought back to 200, by adding flies which up to that time had been in bottles of initial density 35 (optimal). Of the flies taken from bottles of initial density 35 to fill the 200 bottles back to a density of 200, each had the tips of its wings clipped off, so that they could be subsequently identified.

One purpose of these experiments obviously is to determine whether the experience of the fly relative to density up to 16 days of age influences its subsequent mortality in conditions of high density. The case stands this way. Flies starting at initial density 200 are allowed to die off in a manner normal for that density till they are 16 days old. Then the survivors are once more submitted to a density of 200 flies per one ounce bottle, the flies to bring back the density to this point being marked (by wing clipping) so that their subsequent mortality may be separately recorded. These added flies are also aged 16 days at the time they are subjected to a density of 200, but during the first 15 days of their lives they have been subjected only to the optimal densities implied by an initial density of 35 flies per bottle.

The experiments were carried out with wild type Line 107 flies, on banana-agar food, at 25° Centigrade, the general technique being identical with that of the earlier experiments described above.

The survivorship distributions are given in Table 7, and the biometric constants for duration of life in Table 8.

These experiments seem to establish the following points:

(1) As in the earlier experiments the mean duration of life is much greater (in these experiments about

double) among flies subjected to the conditions implied by an initial density of 35 flies per one ounce bottle than among flies subjected to the conditions implied by an initial density of 200.

TABLE 7  
SURVIVORSHIP DISTRIBUTIONS FOR CHANGING DENSITY OF POPULATION

Age in days	A Controls at initial density 35			B Controls at initial density 200	C Changed density			
	Wings not clipped	Wings clipped	Total		Wings not clipped. Started at density 200. Returned to density 200 at 16 days of age	Wings clipped. Started at den- sity 35. Went to density 200 at 16 days of age		
1- 3	.....	.....	1000	1000	1000	.....	1000	.....
4- 6	.....	.....	994	968	925	.....	988	.....
7- 9	.....	.....	988	895	873	.....	974	.....
10-12	.....	.....	981	742	731	.....	966	.....
13-15	.....	.....	954	493	529	.....	951	.....
16-18	1000	1000	917	381	341	1000	927	1000
19-21	962	952	878	316	126	368	514	555
22-24	906	887	822	254	43	125	275	297
25-27	830	762	729	174	31	92	204	220
28-30	708	632	613	136	18	53	146	157
31-33	623	550	536	119	13	39	114	123
34-36	472	450	422	86	9	26	75	81
37-39	382	385	352	69	7	20	57	62
40-42	288	273	257	51	3	10	38	41
43-45	236	221	209	32	1	3	29	31
46-48	189	182	170	25	0	0	18	19
49-51	146	130	126	18	.....	.....	12	13
52-54	123	117	110	16	.....	.....	10	10
55-57	66	82	68	9	.....	.....	0	0
58-60	57	56	52	9	.....	.....	.....	.....
61-63	38	26	29	6	.....	.....	.....	.....
64-66	14	26	19	6	.....	.....	.....	.....
67-69	9	17	12	6	.....	.....	.....	.....
70-72	9	17	12	3	.....	.....	.....	.....
73-75	5	9	6	3	.....	.....	.....	.....
76-78	0	0	0	0	.....	.....	.....	.....
Absolute no. of flies .....	212	231	483	771	892	304	737	681

TABLE 8  
BIOMETRIC CONSTANTS FOR DURATION OF LIFE WITH CHANGED DENSITY OF  
POPULATION

Group	Mean (days)	Standard deviation (days)	Coefficient of variation (per cent.)
A. Controls at initial density 35.			
1. Total distribution .....	33.27 $\pm$ .41	13.27 $\pm$ .29	39.87 $\pm$ .99
2. Mortality from 16 days of age on. Wings not clipped .....	35.68 $\pm$ .54	11.67 $\pm$ .38	32.70 $\pm$ 1.18
3. Mortality from 16 days of age on. Wings clipped .....	34.82 $\pm$ .54	12.28 $\pm$ .39	35.26 $\pm$ 1.24
B. Controls at initial density 200.			
1. Total distribution .....	16.95 $\pm$ .27	11.23 $\pm$ .19	66.25 $\pm$ 1.56
C. Changed density.			
1. Wings not clipped. Sub- jected to density 200 at age 1 day, and again at age 16 days. Mortality from 16 days on .....	19.71 $\pm$ .17	4.38 $\pm$ .12	22.24 $\pm$ .64
2. Wings clipped. Sub- jected to density 35 at age 1 day, and to density 200 at age 16 days. Mortality from 16 days on .....	22.83 $\pm$ .19	7.27 $\pm$ .13	32.56 $\pm$ .66
3. Total distribution. Mor- tality from emergence on .....	13.44 $\pm$ .14	6.05 $\pm$ .10	45.02 $\pm$ .85

(2) When flies which have lived the first 15 days of their lives under the conditions implied by an initial density of 35 are on the 16th day of their age submitted to a density of 200, and live out the remainder of their lives under the conditions implied thereby, their average duration of life is reduced in these experiments from the 34 or 35 days which it would have been had they stayed in the bottles of initial density 35, to 22.83 days. This result shows that crowding produces a heavy increase in mortality even though it occurs as late as 16 days of age.

(3) Flies which have lived for the first 15 days of their lives under the conditions implied by an initial density

of 200, and then at the age of 16 days are again subjected to a density of 200, have a significantly shorter duration of life than do their companions in the same bottles who spent the first 15 days of their lives in bottles of initial density 35. The difference is  $22.83 \pm .19 - 19.71 \pm .17 = 3.12 \pm .25$  days. This difference is more than 12 times its probable error. It may be taken as probable to the point of practical certainty that excessive crowding in early life deleteriously affects the survivors of 16 days of age, so that they are significantly less resistant to the effects of heavy crowding again at that time than are flies which lived at optimal densities in early life.

(4) Clipping the wings to the extent which was practiced in these experiments for purposes of identification did not significantly affect duration of life ( $35.68 \pm .54 - 34.82 \pm .54 = 0.86 \pm .76$ , an obviously insignificant difference).

The course of events in the experiments is shown in Figs. 9 and 10 which are plotted from the data of Table 7.

Figure 9 shows that when the flies which had spent the first 15 days of life in bottles of initial density 35 were crowded up to density 200 their survivorship curve at once dropped to the level of the 200 density control bottles and continued nearly to the end of life parallel and very close to that curve. Those that started at density 200, when brought back to this density at 16 days of age, sustained at once a great increase in mortality, and their survivorship curve dropped and stayed well below the 200 density control curve, throughout the remainder of the life span.

Figure 10 shows clearly, in the first place, that clipping the tips of the wings to the extent practiced in these experiments did not *per se* alter the rate of mortality in those flies that remained in bottles of initial density 35 throughout life. The two upper survivorship curves could hardly be closer together than they are. Furthermore this diagram shows in a striking way the fact that flies which had spent the first 15 days of life at high

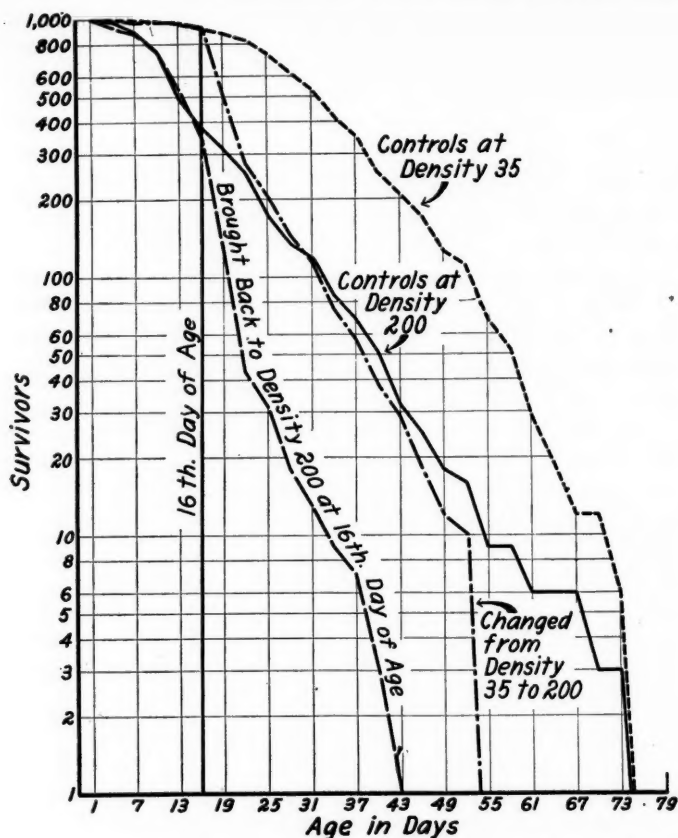


FIG. 9. Survivorship curves showing the effect of changing density of population at the 16th day of age. Explanation in text.

densities were much less able to withstand the deleterious effect of renewed high densities, beginning at age 16 days, than were flies which had spent the first 15 days of life at optimal densities.

#### Discussion

There is a small literature of careful experimental studies on the effect of density of population upon different biological processes, notably growth. The more important papers and books are those of Semper (38),

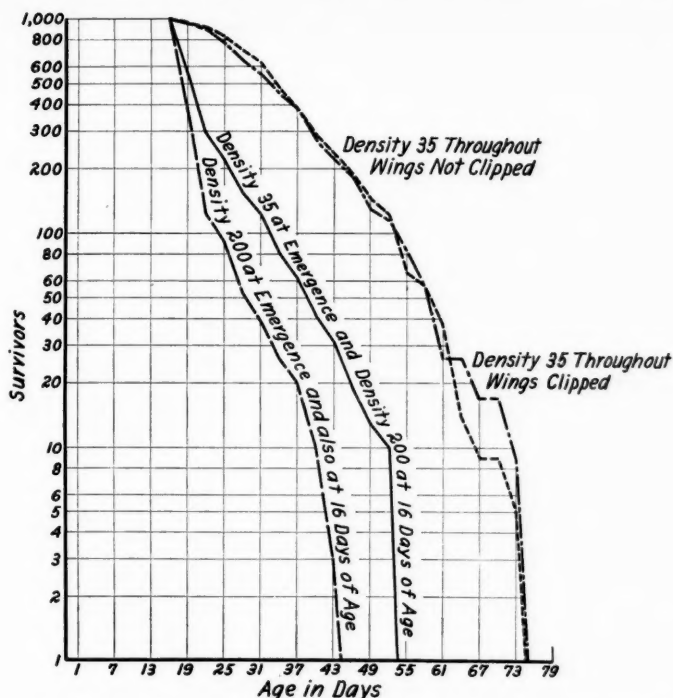


FIG. 10. Survivorship curves starting with 1,000 individuals at age 16, showing the effect of prior history upon duration of life after that age.

Bilski (39), Drzwina and Bohn (40), Pearl and Surface (41), Pearl and Parker (42, 80), Pearl, Allen and Penniman (79), Pearl (81), de Varigny (82), Robertson (86, 87), Greenleaf (88), Cutler and Crump (89, 90, 91), Legendre (92), and Willem (93).

There is also a statistical literature on the correlation between density of population and mortality in human populations. Here the important references are Farr (35), Brownlee (36, 37), LeBlanc (83), Mortara (84), Bowley (85).

There can be no question that the degree of crowding of organisms together in spatially limited "universes" has a profound effect upon various vital processes. In

the present paper we have shown that different degrees of density of population have associated with them marked and orderly differences in the duration of life of *Drosophila melanogaster*. In all of our experimental work we have found no other environmental factor which produces such marked alterations in life duration. The finding that high degrees of crowding shorten life (increase the rate of mortality) is the result to be expected on general grounds. But the further clean-cut evidence which we have presented for normal wild-type *Drosophila* that minimal population densities are not optimal for duration of life is a wholly unexpected result. We were at first skeptical about its truth. But the never-failing appearance of this phenomenon in all the repeated experiments which we have made has finally forced us to accept it as a fact, at least under all the different experimental conditions which we have so far devised.

The experiments here reported also suggest that there is probably a limiting asymptote to the effect upon duration of life which can be produced by increasing the degree of crowding of the flies in the bottle. After a density of 200 flies per one ounce bottle is reached further increases in density of population produce but slight further reductions in mean duration of life.

We have further shown that the most marked effect of density of population upon life duration is produced early in life, though excessive crowding can immediately increase the death rate at later periods of life, and by inference at any age. Furthermore it appears that the amount of shortening of life produced by crowding at any age is influenced by the previous history of the flies relative to density of population. This suggests that there is a deleterious biological effect of crowding in early life even upon those flies that do not immediately die as a result of it, and that this effect endures for at least the first 15 days of life.

Having established the fact that there is a definite, characteristic and systematic effect of density of popu-



lations upon the duration of life, the further problem then presents itself of finding out the biological processes through which this effect is produced. In most of the previous work on the subject, which has been done chiefly with organisms living in a fluid medium, the tendency has been to attempt to explain the effect of density of population on vital processes as the result of the accumulation in the medium either of metabolic waste products or of mysterious chemical substances, scientifically denominated as "X substances." But however true (or untrue) this type of explanation may be for water-inhabiting forms, it is clearly very dubious for a form like *Drosophila* living in bottles in which there is a free circulation of air (through perforated stoppers).

Our studies of the matter indicate that, for *Drosophila*, the biological explanation of the effects of density of population lies in an entirely different direction. The nature of this explanation can not be gone into here, but we expect in a later paper to discuss it fully, with a detailed presentation of the observational and experimental evidence upon which it is based.

## LITERATURE CITED

(The plan of numbering citations is explained in the second of these studies, AMER. NAT., Vol. 56, p. 174.)

78. Pearl, R., and W. B. D. Penniman. "Culture Media for *Drosophila*." I. "Changes in Hydrogen Ion Concentration of the Medium." AMER. NAT., Vol. 60, pp. 347-357, 1926.
79. Pearl, R., Allen, A., and W. B. D. Penniman. "Culture Media for *Drosophila*." II. "A New Synthetic Medium and its Influence on Fertility at Different Densities of Population." *Ibid.*, pp. 357-366, 1926.
80. Pearl, R., and S. L. Parker. "Experimental Studies on the Duration of Life of *Drosophila melanogaster* in the Complete Absence of Food." *Ibid.*, Vol. 58, pp. 193-218, 1924.
81. Pearl, R. "The Biology of Population Growth." New York (Alfred A. Knopf, Inc.), 1925. Pp. xiv + 260.
82. de Varigny, H. "Recherches sur le nanisme expérimental. Contribution à l'étude de l'influence du milieu sur les organismes." *Jour. de l'Anat. et Physiol.*, T. 30, pp. 147-188, 1894.
83. LeBlanc, T. J. "Density of Population and Mortality in the United States." *Amer. Jour. Hyg.*, Vol. 4, pp. 501-558, 1924.

84. Mortara, G. "Lo sviluppo delle grandi città Italiane." *Rivista d'Italia*, 1907. Pp. (of reprint) 1-57.
85. Bowley, A. L. "Death-rates, Density, Population and Housing." *Jour. Roy. Stat. Soc.*, Vol. 86, pp. 516-539. Discussion, pp. 539-546, 1923.
86. Robertson, T. B. "Experimental Studies on Cellular Multiplication." I. "The Multiplication of Isolated Infusoria." II. "The Influence of Mutual Contiguity upon Reproduction Rate and the Part played therein by the 'X-Substance' in Bacterised Infusions which stimulates the Multiplication of Infusoria." *Biochem. Jour.*, Vol. 15, pp. 595-619, 1921.
87. *Id.* "Reproduction in Cell-communities." *Jour. Physiol.*, Vol. 56, pp. 404-412, 1922.
88. Greenleaf, W. E. "The Influence of Culture Medium and Cell Proximity on the Rate of Reproduction of Infusoria." *Jour. Exper. Zool.*, Vol. 46, pp. 143-167, 1926.
89. Cutler, D. W., and L. M. Crump. "The Rate of Reproduction in Artificial Culture of *Colpidium colpoda*." *Biochem. Jour.*, Vol. 17, pp. 174-186, 1923.
90. *Id.* Part II. *Ibid.*, pp. 878-886, 1923.
91. *Id.* Part III. *Ibid.*, Vol. 18, pp. 905-912, 1924.
92. Legendre, E. "Recherches sur le nanisme expérimental. Influence des excreta." *Arch. de Zool. Exp. et Gén.*, 4<sup>e</sup> Sér., T. 8, *Notes et Revue*, pp. LXXVII-LXXXIV, 1908.
93. Willem, V. "Observations sur la respiration cutanée des Limnées et son influence sur leur croissance." *Bull. de l'Acad. Roy. de Belgique*, 66<sup>me</sup> Année, 3<sup>me</sup> Sér., T. 32, pp. 563-577, 1896.

## SEX AND SEX-DETERMINATION IN THE LIGHT OF OBSERVATIONS AND EXPERIMENTS ON DIECIOUS PLANTS<sup>1</sup>

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ORDINARY hereditary phenomena, as exhibited in both the plant and animal kingdoms, resolve themselves into a multitude of physiological and morphological characters of the most diverse nature. In each group and in each species peculiar characters appear and are handed on from generation to generation. Thus when one compares the peculiarities of a dog and a hemp plant there is little correspondence after one passes beyond the most fundamental cell structures and the fundamental physiological processes that they may have in common. These fundamental structures and functions, as protoplast, nucleus, chromosomes, respiration, assimilation, cell division, etc., are not in themselves considered as special categories, but are assumed to be necessary bases and primary properties which at least all the higher organisms have in common. When one attempts to compare the calyx of the hemp with any corresponding part of a dog one is immediately confronted with the fact that there is no correspondence. The same is true in respect to an enormous number of organs, tissues, chemical bodies and functions of the two organisms. It is thus evident that although they are alike in many ways, each one has a multitude of hereditary peculiarities which can not be duplicated in the other.

But when we compare the two organisms as to their sexual natures we again find a most remarkable agreement although the secondary sexual characters expressed

<sup>1</sup> Papers from the Department of Botany, The Ohio State University No. 202. Presented, by invitation, at the meeting of the Ohio Academy of Science. April 16, 1927.

are as diverse in nature as their other hereditary characters. Both have a fundamental duality which is manifested in the secondary sexual characters as well as in the primary sexual characters of the gametes. Both have the same peculiar, specific sexualization of their chromosome sets leading on to synapsis at the proper point of the life cycle. The same type of specialization appears in the sexual cells, the same remarkable properties of attraction and fusion resulting in a zygote, the same phenomena of duality wherever sexuality is involved.

Sexual states and sexual conditions are therefore not to be regarded as coming into the domain of ordinary Mendelian heredity, but rather one perceives that such heredity is the result of sexuality. Furthermore, changing sexual states cause changed hereditary expressions of a very large number of Mendelian hereditary factors. To any one with the most elementary knowledge of cytology and the life-cycle of organisms it is evident that neither the original sex-determinations nor the later sex-reversals, in the vast majority of the different types of organisms, have any correspondence with segregations and aggregations of chromosomes which are at present regarded by geneticists as the bearers of hereditary factors, and their movements in reduction and fertilization as the direct cause of the ordinary Mendelian phenomena.

But if sexuality is not directly involved with Mendelian heredity, with the shifting of chromosomes, what is it? Experiment and observation have gone far enough to show that sex is due to some fundamental physical or chemical state in the living protoplast either of the protoplasm itself or of its inclusions. This state may be positive or female (+), negative or male (—), or completely neutral. The positive or negative condition may be of varying degrees of intensity and persistency. The sexual state is apparently produced or determined by a metabolic condition or level in the cell. The change from one sex to the other or to neutrality, whether of cells of the body or of gametes, is then dependent on an increas-

ing or decreasing metabolic gradient, either directly or in relation with other gradients, as gradients of determination, of maturity, of senility, etc. Sexuality, both male and female, also manifests itself in several different and distinct states. The most obvious are the secondary and primary sexual states. The normal sexual states have a direct influence on the hereditary expression of numerous factors, so that when they are present secondary sexual characters or sex dimorphisms appear. Such secondary states, however, do not involve the cell in any special attractive properties. All that happens, so far as we know at present, is a particular mode of hereditary expression. Under the female or plus (+) sexual state the expression is of one character, under the male or minus (—) state the expression of the same protoplast or of its direct vegetative descendant is of a different character; and thus we speak of a female and male duality. A neutral state may of course give still another type of expression.

The primary sexual state may manifest itself in cells which show no sexual dimorphism whatever or in cells which show the most extreme type of sexual dimorphism. When the primary sexual state is present the cells show the remarkable property of attraction and fusion and the production of one unitary protoplast from two. In some organisms as in the higher fungi and in some species of *Oedogonium* a partial primary state, preceding the complete primary state, is developed, but this does not end for the time being in a fusion of the nuclei. In *Oedogonium* there is not even cytoplasmic fusion; in the higher fungi cytoplasmic fusion is accomplished. At a later stage a complete primary sexual state is developed, when the protoplast acquires the property of complete fusibility. At every reduction division there is also a plus (+) and minus (—) sexualization of the synaptic pairs of chromosomes which results in a temporary union of the two bodies, normally without a commingling of substance. This may be regarded as a special case of pri-

mary sexualization, which occurs at a definite point in a determinate gradient of the organism. The chromosome pair for the time being form a unitary system.

Now we can advantageously represent the activities and movements of sex determinations and changes by the simile of a poised balance. It must be recalled that in cases of the normal sexual condition of higher organisms, the primary sexual states follow secondary sexual states and in case incomplete sexual states are present in the life cycle these precede the complete primary sexual states. In the normal processes of sexualization, therefore, the progression seems to be in the order—secondary sexual states, primary sexual states; or secondary sexual states, incomplete primary sexual states, complete primary sexual states. Thus it is possible that all sexual states whether primary or secondary are simply manifestations or progressive degrees of the same fundamental condition, or that the secondary condition is a step from the neutral, vegetative condition toward the primary condition.

Our sexual process balance (Fig. 1) can then be represented to work as follows: The left hand arm and pan may represent the plus (+) or female side, the right

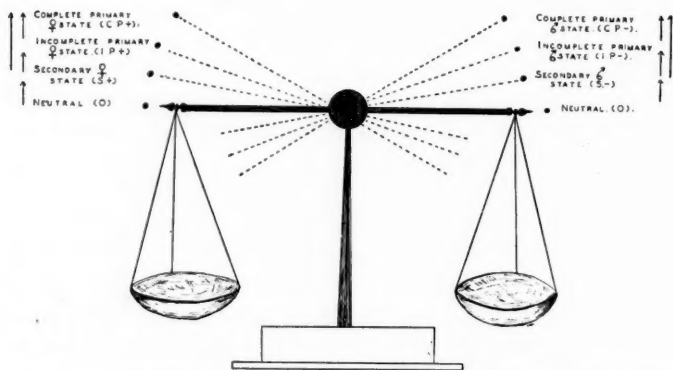


FIG. 1. Diagram of a balance to illustrate the relation of physiological conditions or gradients to the determination and succession of sexual states in the ontogeny.

hand arm and pan the minus (—) or male side. When the balance is horizontal the pans are at zero and we have a purely neutral, vegetative condition. If the functional activity accumulates weights on the left pan to a certain degree, a secondary male state will develop and secondary male characters become manifest. With a greater accumulation, which would only occur at a certain point of the ontogenetic gradient, a primary male state will develop, accompanied by primary male characters, or in the special cases this will be preceded by an incomplete primary male state. If the functional activity accumulates weights on the right pan to a certain degree, a secondary female state will develop and secondary female characters become manifest. With a greater accumulation a primary female state will develop, accompanied by primary female characters, or these may be preceded by an incomplete primary female state. With a changed metabolism and a changed gradient or physiological condition the balance will fall in the opposite direction and sex reversal will result in the given tissue.

At present we can reasonably assume, as a temporary mode of thought at least, that sexuality or cells, tissues, organs and individuals is due to some state or condition, related to chemico-electrical phenomena, which arises in the protoplast through the influence of metabolic gradients and can thus be changed from one condition to the other by the shifting of the functional or gradient conditions through environmental control. The duality of sexuality is apparently a manifestation of the same duality which we perceive in the non-living, as electricity, magnetism, ionization and the like. It is a manifestation of something that we can call positive (+) and negative (—).

The foregoing views and deductions rest on the evidence outlined below, accumulated by the writer and others in recent years. The general evidence that sex is not a phenomenon of heredity lies in the fact that in the vast majority of types of species of plants and animals the time of sex determination or of sex reversal

does not correspond with the shifting of the chromosomes. In apparently none of the animals, in none of the heterosporous plants and in very few of the homosporous plants does any change in sex, whether secondary or primary, follow the reduction division. In none of the hermaphroditic plants or animals, in very few homosporous plants, whether Bryophytes or homosporous Pteridophytes, and in none of the heterosporous sporophytes of all the higher plants, except the comparatively few diecious species, is there a correspondence between sex determination and the aggregation of chromosomes in fertilization. Moreover, in any number of cases a cell lineage is known to pass successively, without any change of chromosomes or hereditary constitution, through all the different sexual states of which the organism is capable.

The evidence from a study of the taxonomy of the higher plants is conclusive that there is no important distinction between hermaphroditism or bisporangiate-ness, on the one hand, and unisexuality or monosporangiate-ness of the individual, whether haploid or diploid, on the other. All along the line the unisexual and the diecious species appear in close relation to a series of hermaphroditic and monecious species simply as the final stage in the evolutionary cycle of sexual progress. In the passage from the condition of bisporangiate flowers to extreme moneciousness and from this condition to complete dieciousness, nothing new appears in any sexual phenomenon whatever, except that the sexual state may be manifest from the beginning and in the entire individual instead of in its special greater or smaller parts, and that in certain species an allosome set may be present in the chromosome complement. And it is plainly evident that sexuality has made allosomes possible as a final step in sexual evolution rather than that allosomes are the cause of sexual phenomena and sex determination.

Since in the final analysis it is apparent that our incorrect and fantastic hypotheses of sex have been based



almost entirely on unisexual species, I have for the most part directed my experimental studies on attempts to show that in diecious species, exactly the same as in monocious species, the individual has all the potentialities of both sexes and that by proper investigations, methods for causing sex reversal can be found with the accompanying expression of sexual characters in both directions. Monocious species are, however, just as important for study and have not been neglected.

1. HEMP (*Cannabis sativa* L.). This is a typical diecious plant with very decided secondary sexual dimorphisms. By using a short-light period together with a substratum rich in nitrogen, over 90 per cent. of the individuals show sex-reversal, the male to the female condition and the female to the male. With a constant environment and a rich substratum, the percentage of reversal is directly proportional to the shortness of the daylight period. In some individuals the reversal comes earlier than in others. In some the reversal is complete and remains thus. But it is entirely improper to give any individual a genetic label in respect to sex based on its performance in the first or normal ontogenetic cycle. The environment can be so manipulated that the differentiation of the individual is overcome and rejuvenation begins a new ontogenetic cycle in which the sex may be completely reversed although the first cycle showed no reversal whatever.

Thus in one experiment, a plant of pure carpellate expression which was passing into senescence was rejuvenated and in its second ontogenetic cycle became pure staminate. After a further vegetative growth it became pure carpellate in expression again and remained so through the third rejuvenation cycle.

From these and other experiments it is established that the homozygous-heterozygous sex-heredity formula is a figment of the imagination based on enormous assumptions. The carpellate plant has all the hereditary poten-

tialities for maleness as well as femaleness and the staminate plant has all the potentialities for femaleness as well as maleness. Then what has determined them as female and male in the first place? From the phenomenon of sex reversal it is evident that we must say that the same cause which operates to change the one sex to the other was operative in the zygote when the original sexual condition was determined. Why should any one ever assume anything to the contrary? Where was there a basis, either in experiment or in general observation of the distribution of male and female characteristics in organisms, for the assumption that the one sex was homozygous for sex factors or sex-producing chromosomes and the other heterozygous?

2. JAPANESE HOP (*Humulus japonicus* Sieb. and Zucc.) The Japanese hop plant is a diecious species belonging to the same family as the hemp. It is reported on good authority to contain allosomes, the homomorphic pair being in the carpellate plant and the heteromorphic pair in the staminate plant. Experiments along the same lines as with hemp resulted in all the staminate plants changing to a greater or less degree to femaleness and nearly half of the carpellate plants to maleness. The allosomes are indicators of sex under the normal conditions of the species. They are not determiners nor even producers of sex in the ordinary sense of the term. The method for reversal employed was evidently not the best one which might be developed. An environment must be found that will reverse the carpellate plants as readily or nearly as readily as the staminate plants. It will be noted that in both the hemp and the hop the single environment produced disturbances that caused the sex to reverse in either direction.

3. JACK-IN-THE-PULPIT (*Arisaema triphyllum* (L.) Torr.). This is a diecious plant of the Aroid family which is a family of mostly monocious plants, the lower

having bisporangiate flowers. Apparently *Arisaema* is the only genus with diecious species. In rich soil, carpellate plants can readily be changed to staminate plants by reducing the water supply and leaf surface. Staminate plants can readily be changed to carpellate plants by keeping the full leaf surface and giving abundant water supply. The sex of the individual can, therefore, be shifted at will, although a very small corm and thus a small nutrient supply and the seedling itself will apparently always for the time being produce a staminate individual. Since *Arisaema triphyllum* produces dichotomous branchings quite commonly, such dichotomous twins become interesting subjects for experiment. Under normal conditions they are not only similar in hereditary expression but are normally identical in sex. A pair of twins still connected by a wide zone of living tissue was manipulated in such a way that the sex of the one was changed from femaleness to maleness while the other was continued in the female condition. Thus a pair of Siamese twins was produced, the one carpellate and the other staminate. In this case the manipulation was simply the use of judgment to produce a proper physiological or nutritive balance that each member would get the proper carbohydrate-nitrogen balance in its part of the system or whatever nutritive balance is actually responsible for the control of the sexual states. I was lucky to succeed in the first trial.

4. GREEN-DRAGON (*Arisaema dracontium* (L.) Schott.). This aroid comes in the same category as *A. triphyllum* but under normal conditions it consists of monocious and staminate individuals, the staminate usually being in a great majority. Treatment similar to that given to Jack-in-the-pulpit results in changing the staminate plants to monocious individuals and monocious plants to staminate individuals. A treatment should be found that would change monocious individuals into pure carpellate individuals as well as into staminate individuals. Plants approaching such a condition have been observed.

5. PURPLISH MEADOW-RUE (*Thalictrum dasycarpum* Fisch. and Lall.). In this species under normal field conditions the individuals are carpellate, staminate and intermediate in varying degrees. In general one can find the same diversity of distribution among the various branches of intermediate individuals as exists among the individuals themselves. The question arises whether the different categories of plants, pure carpellates, pure staminate and the intermediate individuals with the various degrees of male and female expression are fixed in their sexual nature and due to various balances of genes or chromosome constitutions or whether they are merely the results of different functional conditions induced by various environments which are responsible for the various differentiation series. A simple experiment showed that the individual differences were not due to different hereditary complexes. Pure carpellate and pure staminate individuals were transplanted from their rich flood-plain habitat to rather dry clayey soil. After two years the carpellate plants had changed to intermediates with staminate flowers and the staminate plants produced carpellate flowers also. The diversity was due to functional states rather than to balances of genes. Had these plants had but a single ontogenetic and reproductive cycle, theorizers might have claimed that the original differences were developed because of differences in hereditary nature.

6. WESTERN PIGWEED (*Acnida tamariscina* (Nutt.) Wood). This characteristically western weed is definitely diecious. Plants raised under ordinary greenhouse conditions in winter showed no sex reversal, but some raised in a nearly pure manure soil, with rather high greenhouse temperatures and the shortest daylight period of winter, did produce a slight amount of sex reversal, the male to the female condition and the female to the male.

7. WHITE MULBERRY (*Morus alba* L.). The white mulberry is a diecious species with sex reversal a common

occurrence. Of 66 trees studied for one year 28 were apparently pure carpellate, 24 were pure staminate and 14 were intermediates. One staminate intermediate had definite reversal of a branch which then continued to be intermediate while the rest of the tree continued staminate. Of the intermediate trees some were decidedly carpellate in expression, having only a catkin or two partly or completely staminate, and some were decidedly staminate with a mere trace of carpellate expression. Some plants were raised from seed obtained from the mulberry tree with the large reversed, intermediate branch noted above, which had developed through open pollination, and of the ten trees brought to maturity seven were carpellate and three staminate. Two of the carpellate plants later in the season showed a very slight staminate development. One of the staminate trees also later produced two catkins with a slight development of femaleness. Thus these plants, although derived from open pollination but having for their two parents a pair of original fathers, showed no abnormal conditions whatever as compared with the generality of white mulberry seedlings derived more normally from an originally female mother and a male father. There is no indication of any kind of homozygousness or heterozygousness in respect to sex.

8. EARLY MEADOW-RUE (*Thalictrum dioicum* L.). This is a rather extremely diecious species, but intermediates can be found of various degrees of intergradation. Some pure staminate and carpellate plants were transferred into normal conditions in the greenhouse. They nearly all died before any change of sex was observed. But one originally pure carpellate plant developed nine stamens at its last blooming period before it died. An intermediate carpellate plant was likewise transplanted to the greenhouse and in less than a year, after two blooming periods, it came out with pure carpellate expression and remained so for five successive blooming periods, only

pure femaleness being in evidence. After this it again developed intermediate flowers. These experiments show how much reliance can be placed on the experimenter's label when a plant is judged by a single performance or even by its entire normal ontogenetic performance and said to be "pure" for sex or an "intergrade."

9. INDIAN CORN (*Zea mays* L.). Under normal conditions corn is a monecious plant of an extreme type. It can be so controlled in its sexual expression that the entire plant becomes carpellate by complete reversal of the sexual expression in the terminal axis to femaleness. The individual can also be controlled so that only the terminal axis develops and thus there is pure male expression, the lateral branches which would produce ears becoming abortive. Whether a method can be devised through which the lateral branches could be definitely thrown to the male state remains to be discovered. With unequal distribution in planting so that some plants are shaded and crowded the general winter short-light conditions will bring out individuals ranging all the way from pure female expression to the normal monecious type. In the extreme carpellate plants the female state appears as early as the third leaf node from the base and remains female throughout the subsequent development. This range of sexual conditions is, therefore, comparable, except that the range is toward the female side, to what is present under normal conditions in such plants like the green-dragon (*Arisaema dracontium* (L.) Schott.), where the individuals run from the normal monecious condition to the purely staminate condition, or to what is occasionally present in the young trees, at least, of the Ohio buckeye (*Aesculus glabra* Willd.), where one can also find individuals ranging from the pure staminate condition to the normal monecious condition and on to some which are decidedly carpellate but not pure in expression.

Thus the three lines of evidence—the non-correspondence of chromosome aggregations and segregations

with the time of sex determination in the great majority of plant and animal types; the taxonomic relation of the unisexual and monosporangiate species to the hermaphroditic and bisporangiate species; and the experimental work on the diecious species themselves—all confirm the statement, which is after all more a statement of fact than theory, that sexuality is physiological and that specific sexuality as maleness and femaleness is not a matter of Mendelian heredity, but is conditioned on functional states.

Besides my own investigations on diecious and monecious plants, there is a large accumulation of direct evidence on the subject, like Mrs. Wuist Brown's studies on sex reversal in the gametophytes of the ostrich fern, Yampolsky's investigations on *Mercurialis*, Gardner's studies on the strawberry, and Maekawa's work on the Japanese *Arisaema*. Our text-books and genetic treatises are cluttered up with numerous assumptions based on other assumptions which are contradicted by established evidence from every side. It is time that biologists do some drastic expurgation of fairy tales from the books they put into the hands of the student, and through which they misguide the public in general, and begin with a wholesome presentation of realities when dealing with the facts and principles of sexuality which are of such great and fundamental significance in biological science.

PAPERS BY JOHN H. SCHAFFNER, DEALING WITH THE  
PROBLEM OF SEXUALITY

1. "The Nature and Development of Sex in Plants." (Presidential address, Ohio Acad. of Sci., Delaware, Ohio, Nov. 26, 1909.) *Proc. Ohio Acad. Sci.* 5: 327-350. 1910.
2. "The Chromosome Mechanism as a Basis for Mendelian Phenomena." *Ohio Nat.* 15: 509-518. 1915.
3. "The Expression of Sexual Dimorphism in Heterosporous Sporophytes." *Ohio Jour. Sci.* 18: 101-125. 1918.
4. "The Nature of the Diecious Condition in *Morus alba* and *Salix amygdaloides*." *Ohio Jour. Sci.* 19: 409-416. 1919.
5. "Complete Reversal of Sex in Hemp." *Science N. S.* 50: 311-312. 1919.



6. "Dieciousness in *Thalietrum dasycarpum*." *Ohio Jour. Sci.* 20: 25-34. 1919.
7. "The Diecious Nature of Buffalo-grass." *Bull. Torr. Bot. Club* 47: 119-124. 1920.
8. "Influence of Environment on Sexual Expression in Hemp." *Bot. Gaz.* 71: 197-219. 1921.
9. "Reversal of the Sexual State in Certain Types of Monecious Inflorescences." *Ohio Jour. Sci.* 21: 185-200. 1921.
10. "Control of the Sexual State in *Arisaema triphyllum* and *Arisaema dracontium*." *Am. Jour. Bot.* 9: 72-78. 1922.
11. "Progression of Sexual Evolution in the Plant Kingdom." *Ohio Jour. Sci.* 22: 101-113. 1922.
12. "The Sexual Nature of Vegetative or Dichotomous Twins of *Arisaema*." *Ohio Jour. Sci.* 22: 149-154. 1922.
13. "Sex Reversal in the Japanese Hop." *Bull. Torr. Bot. Club* 50: 73-79. 1923.
14. "Observations on the Sexual State of Various Plants." *Ohio Jour. Sci.* 23: 149-159. 1923.
15. "The Influence of Relative Length of Daylight on the Reversal of Sex in Hemp." *Ecology* 4: 323-334. 1924.
16. "The Time of Sex Determination in Plants." *Ohio Jour. Sci.* 23: 225-240. 1923.
17. "Expression of the Sexual State in *Sagittaria latifolia*." *Bull. Torr. Bot. Club* 51: 103-112. 1924.
18. "Experiments with Various Plants to produce Change of Sex in the Individual." *Bull. Torr. Bot. Club* 52: 35-47. 1925.
19. "Sex Determination and Sex Differentiation in the Higher Plants." (Symposium on Sex Determination and Sex Differentiation presented before the meeting of the American Society of Zoologists at Washington, D. C., Dec. 31, 1924.) *AM. NAT.* 59: 115-127. 1925.
20. "The Influence of the Substratum on the Percentage of Sex Reversal in Winter-grown Hemp." *Ohio Jour. Sci.* 25: 172-176. 1925.
21. "Synopsis considered as a Sexual Phenomenon." *Ohio Jour. Sci.* 25: 183-189. 1925.
22. "Questionnaire on Certain Facts bearing on the Theory of Sexuality and Chromosome Constitution." *Science N. S.* 63: 384-385. 1926.
23. "The Nature and Cause of Secondary Sexual States with special reference to *Typha*." (Invitation paper read at the joint meeting of Section G (Botanical Sciences), American Asso. Adv. of Sci., The Botanical Soc. Am., the Am. Phytopathological Society, and the Am. Soc. Plant Physiologists. Kansas City, Dec. 29, 1925.) *Bull. Torr. Bot. Club* 53: 189-208. 1926.
24. "Siamese Twins of *Arisaema triphyllum* of Opposite Sex Experimentally induced." *Ohio Jour. Sci.* 26: 276-280. 1926.
25. "Sex-limited Characters in Heterosporous Sporophytes." *Ohio Jour. Sci.* 27: 19-24. 1927.
26. "Sex-limited Characters and Allosome-linked Heredity." *Ohio Jour. Sci.* 27: 105-126. 1927.



# A FACTORIAL ANALYSIS OF CERTAIN SHAPE CHARACTERS IN SQUASH FRUITS

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## INTRODUCTION

As to just how far shape in organisms is the resultant of genetic factors which determine growth in particular dimensions and how far it depends on actual factors for shape which correlate growth in one dimension with growth in another and thus determine the form which develops, are open questions. If such shape factors really exist, an understanding of them is of considerable importance not only for themselves but for the part which they must necessarily play in the inheritance of many quantitative traits.

Mendelian analyses of characters involving shape merely in its qualitative aspect have frequently been made, as in the cases of capsule shape in *Bursa*, comb form in poultry, wing and eye shapes in *Drosophila* and very many others. More detailed studies, involving measurement of dimensions and calculation of ratios or indices between them, in parents and offspring, have less often been pursued, but it is evidently by these more precise and quantitative methods that the problems of the inheritance of organic form can best be solved. The present paper is based on a study of this sort dealing with fruit shape in *Cucurbita Pepo*, and presents evidence that the inheritance of certain shape types, at least, may best be understood on the hypothesis that genetic factors for shape are operative, and that they behave in typical Mendelian fashion.

In 1910 Emerson (1910) reported the results of a cross of an elongate or "crookneck" squash and one of the flat, disc or "scallop" types in which he measured the

length-width index in parents,  $F_1$  and  $F_2$ . He found that the variability of the shape index of the  $F_1$  was essentially the same as that of the parents but that it increased markedly in the  $F_2$ , thus indicating that multiple factors controlling the shape of the fruit were operative. He presented no data as to the classification of the  $F_2$  for shape. Comparatively few quantitative studies of the inheritance of shape have since been made, but notable among these is the work of Leake (1911) on leaf-lobe shape in cotton, where the operation of a single Mendelian factor-pair was demonstrated. In most cases where the inheritance of carefully measured shapes has been studied, no well-marked segregation of types in  $F_2$ , upon which a factorial analysis could be based, has been observed.

In 1922 the author (1922) reported the results of crosses between flat-fruited and spherical-fruited pure lines of *Cucurbita Pepo* in which a sharp 3:1 segregation for fruit shape occurred in the  $F_2$  and in which the difference between the lines could best be interpreted as due to a single genetic factor. Attention was called to the much greater complexity of shape inheritance in certain other crosses. These investigations have been continued and a very considerable body of facts as to the inheritance of various types of fruit shape in *Cucurbita* has been amassed. Some of the results are complex and still difficult of analysis. Others, such as those previously reported and those discussed in the present paper, seem to be relatively simple.

#### METHODS OF STUDY

Over thirty essentially homozygous lines of summer squash have been isolated, many of which have been inbred every season since 1916. The parent types in the crosses here reported were all inbred for at least seven generations before the crosses were made. All plants were grown under as uniform conditions as possible. A single typical fruit was measured on each plant. This

fruit was split open longitudinally (on the axis of the ovary from receptacle to style), one of the halves placed, cut surface down, on a sheet of heavy paper, and its outline traced in pencil. The length of the fruit, on the axis from stalk to corolla scar, and the width at right angles to this axis and at the widest point, were measured. The shape index was determined by dividing the longer of these two dimensions (length and width) by the shorter. This is evidently the most satisfactory way to indicate differences in shape, for some types of fruit are very much longer than wide and others very much wider than long, and the determination of the index by a division of length by width (or *vice versa*) in all cases would result in spreading out one type over a considerable range of indices (from 1.0 to 4.0 or 5.0) and bunching the other in a very limited range (from zero to 1.0). If we regard the isodimensional, essentially spherical, fruit as the basic type and then measure, by equal degrees, the extent of flattening, on the one hand, or of elongation, on the other, we arrive at indices which, when plotted graphically both ways from 1.0, or analyzed biometrically, give us a much more accurate picture of shape differences than could be obtained by the usual method of determining indices. With such a shape index for a given plant must then evidently be included a notation stating which dimension is the greater, and this has been done by adding *W* or *L*, indicating that the width or the length, respectively, is the longer dimension. A typical elongate squash like the "crookneck" might thus have an index of 3.0 *L*, and a typical flat or disc squash one of 3.0 *W*.

In classifying a population for graphic or biometric purposes, the logarithm of the index rather than the index itself was used. This probably gives a more accurate picture of fruit shape in a population because it brings in the more extreme types closer to the mean, a desirable result since a given unit of dimensional change will tend to have a more profound effect on the index the higher the index is. If we imagine, for example, an

elastic sphere 10 cm. in diameter as being progressively flattened, the change produced in its shape index by each successive centimeter by which its axis is shortened will be progressively greater.

### RESULTS

As previously reported, a pure line of spherical-fruited squashes, line 103, with an index of about 1.0, when crossed with various lines of discs having indices of from 2.0 *W* to 3.5 *W* gave an  $F_1$  essentially like the disc parent and an  $F_2$  with sharp segregation into approximately 3/4 discs and 1/4 spheres. The difference between this particular sphere and the ordinary disc type seems here to be due to a single shape factor. Evidence that this result does not depend on the segregation of dimensional factors merely, or on linkage between dimensional factors, was presented in a previous paper. This conclu-

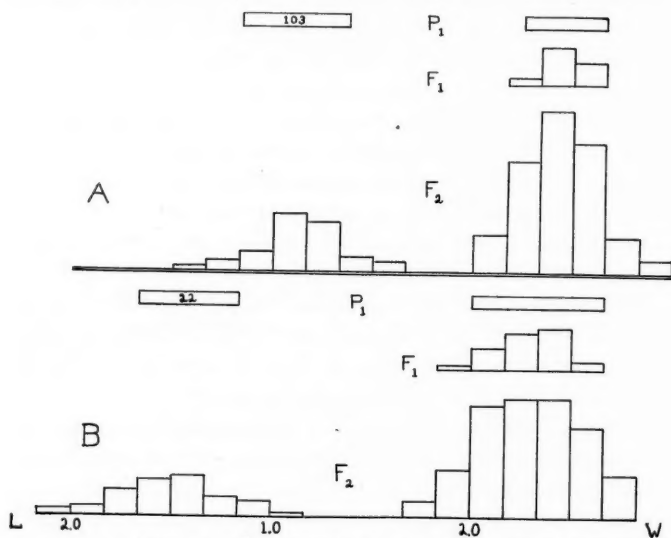


FIG. 1. *A*, shape indices of parents,  $F_1$  and  $F_2$  in a cross between line 103, at left, and a disc type, at right. *B*, the same for a cross between line 22, at left, and disc type, at right. Elongation is plotted to the left of 1.0 and flattening to the right.

sion has been strengthened by all later work and will be discussed more fully in a later publication. The shape indices of parents,  $F_1$  and  $F_2$  of this cross, are shown in Fig. 1A. The ordinary range of fluctuating variability for the parent type is indicated, the mid-point on the band being the mean for the type. In the particular cross here presented (line 103  $\times$  line 13) the  $F_2$  consisted of 296 individuals, of which 220 or 74.3 per cent. were discs and 76 or 25.7 per cent. were spheres, a ratio of 2.90:1.

In our cultures there early appeared a number of other lines which were intermediate between the disc type and the elongate type. One of these, line 22, came from commercial seed under conditions which led to the suspicion that it was a segregate from a natural cross of "crook-neck" with disc. It was rather variable at first but after a few generations of self-fertilization has become definitely fixed as a rather elongate spheroid with its widest diameter nearer the stalk than the corolla end and a mean index of about 1.3 *L*. In crosses with the flat or disc type this behaves as a recessive and gives a clear 3:1 ratio in the  $F_2$ . In Fig. 1B the shape indices of parents,  $F_1$  and  $F_2$  of a cross between line 22 and some disc types are shown. The combined  $F_2$  populations gave 161 discs and 48 elongate spheroids with essentially the same mean as line 22, a ratio of 3.35:1. Between line 22, therefore, and the disc types of squash, just as between line 103 and the discs, there is evidently a single factor difference.

In 1923, lines 103 and 22 were crossed and the  $F_1$ , grown in 1924, proved to be typically *disc* in shape with an index of about 2.5 *W* (Fig. 2). The  $F_2$  grown in 1925 showed marked segregation, roughly trimodal in type. More than half of the  $F_2$  were disc in shape essentially like the  $F_1$ , though somewhat less flat, but there were a very considerable number of plants resembling lines 103 and 22, and a much smaller proportion markedly more elongated than either of these lines. The shape indices of the combined  $F_2$  populations from this cross are shown in Fig. 2. Of the total population of 276,

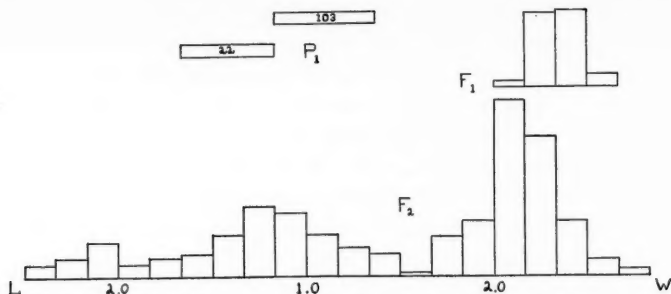


FIG. 2. Shape indices of parents,  $F_1$  and  $F_2$  in a cross between lines 103 and 22, showing the 9:6:1 ratio in  $F_2$  (right to left).

154 or 56 per cent. had an index of 1.5  $W$  or flatter, and were essentially disc in shape, and 122 or 44 per cent. were spherical or longer, a close approach to the familiar 9:7 ratio. This, and the marked difference of the  $F_1$  from both parent types, at once suggested the operation of complementary factors, each one of which alone produce a spherical or nearly spherical fruit, but which together determine the much flatter disc type. This explanation, however, does not account for the small but invariably present group of plants with markedly elongate fruits. Their occurrence in approximately 1/16 of the  $F_2$  suggested that they might be double recessives. Their marked difference from the parent spherical types further suggested that each of the factors possessed, respectively, by line 103 and line 22 were dominant over the elongate fruit shape, tending to flatten it from an elongate to an approximately spherical form; that these two flattening factors were distinct, and that their flattening effect was *cumulative*, so that in the  $F_1$  of a cross between the two lines a much flatter squash than either (the disc) was produced.

This hypothesis of two independent flattening factors, cumulative in their effect, explains the production of a flat-fruited  $F_1$  from a cross between two spherical-fruited plants, and the general appearance of the  $F_2$ , especially the presence of a small group of much elongated fruits.

If the hypothesis is true, one should be able to predict from it (a) the exact ratio of the  $F_2$ ; (b) the appearance of the offspring of backcrosses between the  $F_1$  and each parent and (c) the appearance of various types of ratios in the  $F_3$ . These tests were applied and the hypothesis seems to meet them very satisfactorily.

(a)  $F_2$  Ratio. If the two dominant cumulative flattening factors are designed as  $A$  and  $B$ , the genotype of line 103 may be written  $AAbb$  and of line 22  $aaBB$ . The  $F_1$  in this case would thus be  $AaBb$ . 9/16 of the segregating  $F_2$  should possess both flatteners and have disc fruits; 3/16 should possess  $A$  but not  $B$ , and 3/16  $B$  but not  $A$ , so that 6/16 should be spheres or elongate spheres; and 1/16 should be the double-recessive, elongate form  $aabb$ . We should therefore expect an  $F_2$  ratio of 9:6:1.

The  $F_2$  shown in Fig. 2 represents the sum of three  $F_2$  populations from the original cross of  $103 \times 22$ , consisting of 276 individuals. If everything flatter than 1.5  $W$  is classed as a disc, everything from 1.5  $W$  to 2.0  $L$  as a sphere or long sphere, and everything longer than 2.0  $L$  as an elongate type, there follow the results set forth in the ensuing tabulation, where the actual counts and those expected on the assumption of a 9:6:1 ratio are compared. The agreement between the theoretical and the actual results is satisfactorily close:

	Disc	Sphere	Elongate
Observed .....	154	101	21
Expected .....	155.25	103.50	17.25

(b) *Backcrosses*. If the genotype of the  $F_1$  is  $AaBb$  the result of a backcross of this  $F_1$  on the parents should give, with line 103, 1/2 discs ( $AB$ ) and 1/2  $Ab$  spheres; and with line 22, 1/2 discs and 1/2  $aB$  spheres. These crosses, unfortunately, were not made with the original  $F_1$ , but the cross was repeated in 1924 and a similar  $F_1$  raised in 1925, which was crossed on the two parental lines with the result shown in Fig. 3. In each case ap-

proximately half of the offspring are discs and half spheres or long spheres (the total count is 52 discs: 49 spheres); and the extracted spheres are essentially like the parent spheres, those coming from the cross of  $F_1$  with line 103 being markedly flatter than those from line 22. These two types of spheres ( $Aabb$  and  $aaBb$ ) should give, when crossed,  $1/4$  discs,  $1/2$  spheres and  $1/4$  elongate fruits. This cross was made during the past season and results will be forthcoming during the present year.

(c)  $F_3$  Progenies. If our hypothesis is correct, the expected phenotypical and genotypical constitution of the  $F_2$  should be as follows:

Discs	Spheres	Elongates
1 $AABB$	1 $Aabb$	1 $aabb$
2 $AABb$	2 $Aabb$	
2 $AaBB$	1 $aaBB$	
4 $AaBb$	2 $aaBb$	

We should have in the  $F_2$  four types of disc-fruited plants genotypically different from each other:  $AABB$ ,  $AaBB$ ,  $AABb$  and  $AaBb$ . When self-fertilized the first type should give nothing but discs; the second and third should give approximately  $3/4$  discs and  $1/4$  spheres, and the last should produce progenies resembling the  $F_2$ , and giving a 9:6:1 ratio. A considerable number of  $F_3$  progenies were grown during the past season from self-fertilized  $F_2$  discs of 1925. The three expected types of progenies were found, and it was possible in every case to assign a given disc-fruited plant to one of the three genotypic groups (doubly homozygous, singly heterozy-

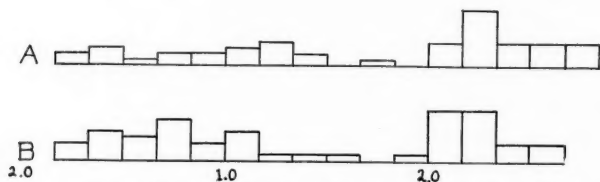


FIG. 3. Shape indices of offspring of backcrosses of  $F_1$  with the two parent types. A,  $F_1 \times$  line 103; B,  $F_1 \times$  line 22.



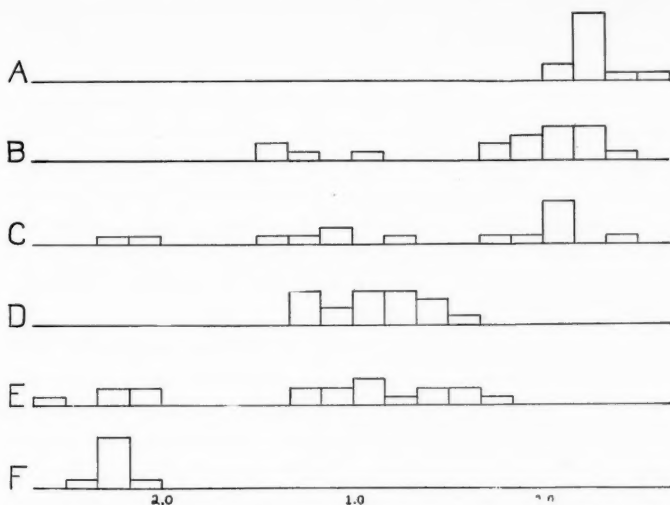


FIG. 4. Shape indices of  $F_3$  progenies from self-fertilized  $F_2$  individuals. *A*, from doubly homozygous disc; *B*, from singly heterozygous disc; *C*, from doubly heterozygous disc; *D*, from homozygous sphere; *E*, from heterozygous sphere; *F*, from elongate.

gous, doubly heterozygous). Typical  $F_3$  progenies from inbred  $F_2$  discs are shown in Fig. 4, *A*, *B* and *C*.

Similarly, there should be four types of spherical-fruited plants in the  $F_2$ :  $AAbb$ ,  $aaBB$ ,  $Aabb$  and  $aaBb$ . The first two of these should produce nothing but spheres when inbred and the last two should give approximately 3/4 spheres and 1/4 elongate-fruited plants. Both these types were clearly distinguishable in the  $F_3$  progenies, and examples of them are shown in Fig. 4, *D* and *E*. Disc-fruited plants should never appear in these progenies and none did so.

The elongate-fruited plants,  $aabb$ , should all be genotypically alike, and should all breed true, never producing other types when self-fertilized. This expectation was fully met, and a typical  $F_2$  progeny from a self-fertilized elongate plant is shown in Fig. 4 *F*.

The cross between these two lines has now been made several times and the  $F_1$  and  $F_2$  generations are entirely

similar to the one recorded above. There are a number of other essentially spherical-fruited types which are related to line 103, either having come from the same original stock or arisen as segregates from crosses of line 103 with discs. None of these, when crossed with each other, gave anything else than spheres. When crossed with line 22, however, they all behaved like line 103.

All the evidence, therefore, seems to support the hypothesis that in this cross the three main shape types are due to two independent pairs of flattening factors, cumulative and essentially equal in their effect, the presence of both dominant allelomorphs determining the disc shape, both recessive ones the elongate shape and the two combinations of one dominant and one recessive the two spherical types, respectively. The results of a number of other crosses can also be explained on the same hypothesis, with certain minor modifications.

The problem is not quite as simple as this, however. In the first place, there is evidence of a number of other factors influencing fruit shape but markedly smaller in their effect. In a number of the  $F_3$  progenies from disc fruited plants, for example, there was a clear segregation into  $3/4$  flat and  $1/4$  less flat, but the mean of both types was markedly flatter than that of the corresponding discs and spheres in the  $F_2$ . This is understandable if we assume that the  $F_2$  parent of this progeny was homozygous for one of these minor flatteners. It will be noted that the  $F_2$  discs are not as flat as those of the  $F_1$ , a fact which can also be explained by the presence in the  $F_2$  discs of recessive segregates of these minor dominant flattening factors, elongating in their effect. This also explains the marked increase in variability of the  $F_2$  discs over the  $F_1$  discs, which should not occur if merely the two major dominant flattening factors were operative. There are considerable differences in variability and sharpness of segregation between the different  $F_3$  progenies, and these differences can be explained by assuming that the parental  $F_2$  plants differed in the extent

of their heterozygosity for these minor flattening factors.

This hypothesis is also inadequate to explain the inheritance of fruit shape in certain other lines in which the shape index is similar to some of those here studied. There are several lines, for example, of elongate-fruited squashes with a shape index of from 2.0 *L* to 3.0 *L*, very similar in appearance to the double-recessive elongate segregates in the cross here reported. Instead of showing a single-factor difference from the two spherical types here mentioned, however, and a two-factor difference from disc-fruited types, these behave in a much more complex fashion. When crossed with spheres and with discs they produce  $F_2$  populations which often show little evidence of segregation and in which comparatively few spheres or discs, respectively, appear. In a subsequent paper evidence will be presented that elongate shapes, in their various degrees, are often due not only to recessive allelomorphs of dominant flattening factors but to a series of factors which inhibit flattening. Undoubtedly there are a large number of these shape factors, perhaps one or more in each chromosome, differing both in the nature and the extent of their expression; and a complete Mendelian analysis of shape is therefore a very difficult task.

#### CONCLUSION

The results here reported may be most simply explained on the hypothesis that fruit shape in the squash is controlled by definite genetic factors for shape inherited in typical Mendelian fashion, which in some way control growth correlation, rather than to factors which control growth in any given dimension. The identification of such factors and the investigation of their expression and interaction is of importance not only because of their influence on the inheritance of various quantitative characters but because of the bearing which they must obviously have on the problems of morphogenesis. Morphology in general must ultimately go beyond the methods of description and comparison and concern itself with the physiological and genetic bases of organic form.

## SUMMARY

(1) Two pure lines of squashes, one essentially spherical and the other long-spherical in shape, when crossed produce an  $F_1$  in which the fruits are much flattened like a typical disc or "scallop" type.

(2) The  $F_2$  of this cross gave approximately 9/16 discs, 6/16 spheres, and 1/16 considerably elongated fruits.

(3) It is suggested that these results are due to the operation of two pairs of Mendelian factors, the dominant allelomorph in each case tending to flatten the shape of the fruit, and the recessive to elongate it, and that their effect is cumulative. One parental sphere has one of the dominant factors, and the other parental sphere the other; the  $F_1$  and the  $F_2$  discs have both of them; the spherical segregates in  $F_2$  have one of them, and the elongate segregates have neither of them.

(4) This hypothesis offers a simple explanation of the  $F_2$  segregation, of the results obtained by backcrosses of the  $F_1$  on both parent types, and of the character of the  $F_3$  progenies.

(5) Evidence is presented that in addition to these two major flattening factors there are a number of minor ones, much less pronounced in their effect and inherited independently of the major ones. Factors inhibiting the flattening effect also probably occur.

(6) The conclusion is justified that shape factors, controlling growth correlations, exist in this material and that they are inherited in typical Mendelian fashion.

## LITERATURE CITED

- Emerson, R. A.  
1910. "The Inheritance of Sizes and Shapes in Plants." *AMER. NAT.* 44: 739-746.
- Leake, H. M.  
1911. "Studies in Indian Cotton." *Jour. Gen.* 1: 205-272.
- Sinnott, E. W.  
1922. "Inheritance of Fruit Shape in Cucurbita Pepo." *Bot. Gaz.* 74: 35-103.

## ADAPTATIONS OF THE BATHYPELAGIC NEMERTEANS

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OF all the groups of organisms which recent investigations have discovered in the deep oceans, probably none have become more widely divergent from their littoral relatives and more particularly adapted for a free-swimming life far beneath the surface of the water than have the nemerteans.

The object of this paper is to call attention to some of these special adaptations, but with particular reference to the material collected by the U. S. F. C. S. *Albatross* in various parts of the Pacific Ocean. The results of a detailed study of this material have been published in monograph form during the past year (Coe, '26).

Although the total number of described species of nemerteans is less than six hundred, they are very widely distributed, being found on the bottoms and along the shores of all the oceans, less commonly in fresh water, and occasionally in moist earth and even in moist cavities on trees. They are most numerous along the sea-coasts of Europe and North America, but relatively few species extend along the bottom into the deep oceans which lie beyond the continental shelves. In the open oceans they are represented by a highly specialized group of the *Hoplonemertea*, constituting the tribe *Pelagica*, with peculiar adaptations for slowly swimming or floating idly in particular water layers which lie at considerable depths. It is with this bathypelagic group that this paper deals.

Forty-seven species of these highly modified forms have been described. These have been grouped in nine families and twenty-five genera. The members of each

family naturally have characteristics peculiar to themselves, but many of them have a great amount of gelatinous tissue, or parenchyma, separating the internal organs and giving the worms a very low specific gravity and in many cases a gelatinous consistency and translucency so commonly found in pelagic organisms (Fig. 3). Ocelli, cerebral sense organs and the other special sense organs of their littoral relatives are rudimentary or entirely wanting and excretory organs have not been discovered in any species.

In external appearance the bathypelagic species show great diversity in size and shape as well as in their degree of translucency and in their coloration. In some of the species the sexually mature worms do not exceed 6 to 10 mm in length; most of the other species range from 20 to 40 mm long, while the largest known specimen of *Dinonermertes investigatoris* was 203 mm long, 56 mm wide and 15 mm thick. The bulk of an individual of the largest form is thus some ten thousand times as great as that of the smallest, comparable perhaps to the relative sizes of the ox and the mouse.

The shape of the body is generally quite different from that of most of the littoral species, the latter being as a rule very slender and often ribbon-like and adapted for burrowing in mud or sand or for creeping along submerged objects. A few of the littoral forms, however, are relatively short, broad and flattened and adapted for creeping beneath stones and in narrow crevices. The bathypelagic forms are nearly all broad and flat (Figs. 1, 3, 4) and adapted for maintaining themselves suspended horizontally at great depths with a minimum of muscular effort.

Correlated with the flattening of the body are frequently lateral outgrowths of the body walls in the form of very thin lateral margins, often forming lateral keels or rudimentary horizontal fins. In many forms, also, the posterior extremity is broadened and extremely flattened to form a well-developed caudal fin, giving the worm a

remarkably fish-like appearance (Fig. 4). In only a few species is the body relatively slender and narrowed at both ends, as is so generally the case in littoral nemerteans.

A few of the more characteristic forms of the body are shown in Fig. 1. It will be observed how well they are

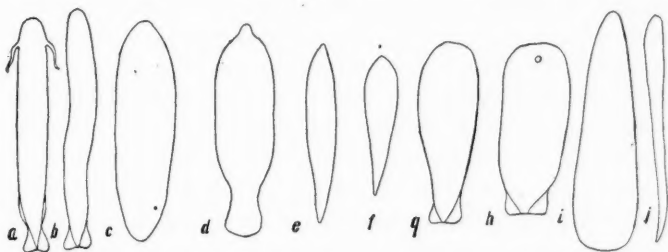


FIG. 1. Outlines of the bodies of several species of bathypelagic nemerteans, showing their adaptive configuration for swimming or floating. *a*, *Nectonemertes mirabilis* Verrill, male; *b*, *N. pelagica* Cravens and Heath, female; *c*, *Planktonemertes agassizii* Woodworth; *d*, *Neuronemertes aurantiaca* Coe; *e*, *Protopelagonemertes hubrechtii* Brinkmann; *f*, *Cunconemertes gracilis* Coe; *g*, *Pelagonemertes brinkmanni* Coe; *h*, *Proarmaueria pellucida* Coe; *i*, *j*, dorsal and lateral views of *Dinonemertes investigatoris* Laidlaw.

adapted for holding themselves in a horizontal position by undulatory movements of the posterior end of the body.

Since the maintenance of their floating position requires more or less continuous undulatory movements of the body it is not surprising to find the musculature highly specialized for this purpose. Instead of having the thick cylinders of circular and longitudinal muscles characteristic of the littoral species, the circular musculature is reduced to a very thin layer of fibers, while the longitudinal musculature is developed into two great horizontal bands, one of which lies on the dorsal side of the body and the other on the ventral side, comparable to the pair of vertical bands which furnish the principal motive power of the fish. The alternate rhythmical contraction of dorsal and ventral muscular bands enables the worm to swim with undulatory movements, the caudal

fin, when present, being the effective organ of propulsion. The degree to which this caudal fin is developed in various species is shown in Figs. 2, 3, 4.

In those species in which the body reaches its extreme degree of thinness there is a great increase in the muscles which pass directly from the dorsal to the ventral side of the body between the intestinal diverticula. The contraction of these muscles brings the dorsal and ventral surfaces of the body closer together and thus makes the worm still more ribbon-like by decreasing its thickness and increasing its length. This dorsoventral musculature reaches its highest degree of development in the posterior part of the body, sometimes producing the extremely thin lateral margins or horizontal fins to which reference has been made (Figs. 2, 4).

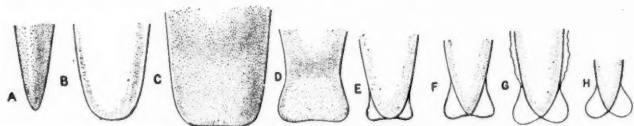


FIG. 2. Diagrams of posterior extremities of the bodies in eight genera of pelagic nemerteans, showing the extent of development of the caudal fin. *A*, *Cunconemertes gracilis* Coe, with slender, subcylindrical posterior end; *B*, *Planktonemertes agassizii* Woodworth, with flattened, rounded extremity; *C*, *Dinonemertes investigatoris* Laidlaw, with extremely flat, truncate end; *D*, *Neuronemertes aurantiaca* Coe, with flat, truncate extremity, but without distinct caudal fin; *E*, *Proarmaueria pellucida* Coe, with slightly developed caudal fin; *F*, *Pelagonemertes brinkmanni* Coe, with fairly well differentiated caudal fin; *G*, *Nectonemertes mirabilis* Verrill, with highly developed caudal fin and pair of lateral fins; *H*, *Balaenanemertes* sp., with extreme development of caudal fin.

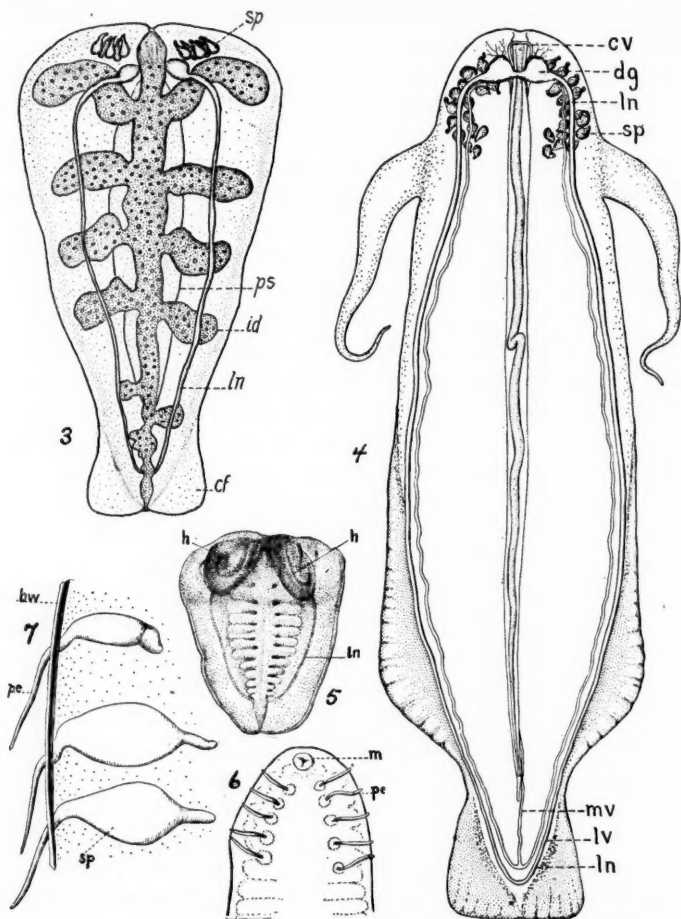
A few forms are provided with appendages in the form of a pair of tentacles near the anterior end of the body (Figs. 1, 4). These tentacles, which are lateral outgrowths of the body walls, occur only in the males of *Nectonemertes* but are found in both sexes of *Balaenanemertes*. In the former genus they reach their full development only with the sexual maturity of the male and are therefore to be looked upon not only as aiding somewhat in the balancing and floating of the body, but



more particularly as appendages for grasping and holding the female until insemination has taken place. For these worms form a sparse population in the depths of the open oceans and any device for holding the sexes in contact, when they chance to meet, until after the eggs have been fertilized may well be looked upon as an important adaptation. In *Plotonemertes* a similar result seems to be accomplished by means of a pair of adhesive organs on the ventral side of the body (Fig. 5). Other curious adaptations in this direction are the situation of the spermaries in or near the head or even in front of the brain in some species (Figs. 3, 4, 6) and specialized musculatures for the forcible discharge of the spermatozoa. In *Phallonemertes* the spermaries are not only provided with a spiral musculature, but the sperm ducts project beyond the body as slender muscular tubes (Figs. 6, 7) which conceivably function as copulatory organs.

In addition to the insurance of fertilization, provision is made for the production of a small number of embryos, each with maximum nutrition. This is accomplished through the absorption of the greater number of the primitive ova to form relatively few of larger size. Not only is the number of ovaries greatly reduced, but only a single one of the numerous primitive ova in each ovary reaches maturity. In one form, *Proarmaueria pellucida*, hermaphroditic gonads occur in addition to ovaries, a condition which would seem to offer certain advantages in a very sparse population.

Many of the species are more or less translucent and some are largely transparent, with only the digestive system and the gonads more opaque and pigmented (Fig. 3). The pigments are of two classes, many species bearing numerous brightly colored oil globules in the walls of the digestive system, while others have in addition a more diffused pigmentation of the whole body. The colors are frequently very bright, shades of yellow, orange, red and scarlet predominating. It must be remembered, however, that since none of the sun's light



FIGS. 3-7. Adaptations of several pelagic species. Fig. 3. *Pelagoneurtes brinkmanni* Coe, indicating the gelatinous consistency of the body, with the deeply colored digestive system showing through the translucent tissues. The spermaries (*sp*) are grouped in two small clusters in front of the brain. Fig. 4. *Nectonemertes mirabilis* Verrill; adult male with fully developed tentacles and cephalic spermaries (*sp*); note fish-like configuration of body, with horizontal and caudal fins. The slender proboscis is shown in the proboscis sheath. Fig. 5. Posterior portion of body of male *Plotonemertes adhaerens* Brinkmann, with pair of glandular organs (*h*), conceivably used in adhering to the female until insemination is completed. Fig. 6. Outline of anterior end of body of male *Phallonemertes murrayi*

rays of these colors reach the depths at which many of the species live the coloration which appears when the animals are brought to the surface must not be assumed to indicate a color adaptation.

In the earlier collections of bathypelagic organisms, which were taken in a dredge or in a net hauled vertically from the bottom, it was impossible to determine the depth from which any given specimen was obtained. By the more recent methods, however, where several nets are placed at different points on a single wire and drawn horizontally at various depths simultaneously the habitat of any species can be determined with considerable accuracy. By such means the approximate bathymetrical range of several species has already been determined. *Nectonemertes mirabilis*, for example, was found by the *Michael Sars* expeditions at numerous stations in the North Atlantic from off the continental shelf near Newfoundland to the similar shelf off the coast of Ireland. Brinkmann ('17) has plotted the distribution of these specimens and finds that the species is more or less closely confined to a water layer having a salinity of somewhat less than 35 per cent. and a temperature of about 4° C. Since this layer is closer to the surface in the western Atlantic than it is at points nearer the coasts of Europe it is to be expected that the depth at which the species is most frequently found will be correspondingly greater towards the east. The data indicate that such is the case, the depth of greatest frequency being about 1,333 meters in the western Atlantic.

Most of the *Albatross* specimens were taken in nets drawn at a depth of about 600 meters beneath the surface in regions where the distance from the surface to the ocean's floor was 2,000 meters or more. We have, unfor-

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Brinkmann, showing the five pairs of external sperm ducts (*pe*) projecting from the ventral surface. Fig. 7. Diagram of three spermaries of same species, showing relation of sperm ducts (penes, *pe*) to body wall (*bw*). Other lettering: *cf*, caudal fin; *cv*, cephalic vessels; *dg*, dorsal ganglion; *id*, intestinal diverticulum; *ln*, lateral nerve; *lv*, lateral vessel; *m*, mouth; *mv*, median dorsal vessel; *ps*, proboscis sheath. (Figs. 5, 6, 7 after Brinkmann, '17.)

unately, no proof as to bathymetrical range of any of these species, and it is quite possible that other species or more numerous specimens of the same species might have been secured if the nets had been lowered to a depth of 1,000 or 1,500 meters.

The absence of appropriate water layers in seas and bays along the continental shelves explains the absence of bathypelagic nemerteans in those regions, while the continuation of these layers beneath the equator allows their free dispersal between the northern and southern hemispheres as well as through all southern oceans. Thus one species, *Dinonemertes investigatoris*, has been found in both the North Atlantic and Indian oceans, *Nectonemertes minima* in the Atlantic from 57° N. to 35° S., *N. pelagica* in the Pacific from 52° N. to 12° S., while *Pelagonemertes rollestoni* extends from the equator in the Atlantic, through the deep water off the western coast of Africa and thence to 4° N. in the Indian ocean and to 50° S. at the region where the Indian and Pacific oceans meet. And it is not improbable that the limits of even this great known range will be extended by future explorations. For up to the present only a minute fraction of the waters of the globe have been explored with apparatus suitable for the collection of bathypelagic organisms.

The bathypelagic nemerteans, therefore, are truly mesoplanktonic organisms, swimming slowly to and fro or floating idly far beneath the surface of the open oceans. For such a life they are admirably adapted, for they are composed so largely of gelatinous tissue that their specific gravity is very low and the configuration of their bodies is such that their position in the depths can be maintained with a minimum expenditure of energy.

#### BIBLIOGRAPHY

Brinkmann, August

1917. "Die pelagische Nemertinen" (monographisch dargestellt).  
Bergens museums skrifte, ny raekke, 3: 194 pp., 16 pls.

Coe, W. R.

1926. "The pelagic nemerteans." Mem. mus. comp. zool. Harvard  
Coll., 49: 245 pp., 30 pls.

## THE SCARABÆUS OF THE ANCIENT EGYPTIANS

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ONE seeks in vain for more than a brief mention of the scarab and its significance in the standard texts on entomology, and apparently only two entomologists, R. P. Dow and J. S. Wade, have gone further and written comparatively extended accounts. For a more or less complete concept one must consult the writings of Egyptologists on both archeology and ancient religion and almost instantly one passes from entomology and is floundering in other fields. It is my purpose, not to flounder any more than can be helped, not to duplicate the works of Dow and Wade except where duplication is unavoidable, but to present briefly the principal theories of archeologists concerning the use and meaning of the Egyptian scarab.

The early Egyptians were surrounded by gods. The sky, the earth, the trees, the animals were peopled by spirits, some friendly and others unfriendly. In the beginning only the ocean of chaos existed, upon which there appeared an egg from which emerged the sun-god. This sun-god gave birth to four children, Shu, Tefnut, Geb and Nut. Shu and Tefnut, who represented the atmosphere, stepped upon Geb and raised Nut up high and so Nut became the goddess of the sky and Geb the god of the earth. Geb and Nut were the parents of the four divinities, Osiris and Isis, Set and Nephthys of the Osiris family, introduced probably by the Libyan people of the first prehistoric age. Osiris, according to an early belief, dwelt in the lower world as king of the dead.

It is said that the various classes of gods in Egypt can be identified with the various races which entered the country. The earliest gods were those coincident with

animals to which the tribes were devoted. Then came the Osiris worshipers, who in turn were followed by the celebrants of cosmic gods from Asia bringing in the second prehistoric civilization, and later abstract gods were introduced. The sun or Ra worship became the important religion of the early kings. Quoting Petrie: "The hour of dawn before sunrise was called 'beholding the beauties of Ra.' At rising, the sun was Her-em-akhti, 'Horus in the horizons.' As he ascended he was Khepera, the god produced to the world. At noon he was Ra, triumphant; as he declined he was Atum, the stifled, till, setting on the horizon, he was again Her-em-akhti, and vanished into the starry Duat." Absent during the night he was Osiris.

The Egyptians, being familiar with the scarabæid beetles, *Ateuchus sacer*, *Scarabæus Aegyptiorum* or related species which are common in Mediterranean countries, and noting the habits of the insects in rolling up balls of dung in which the female encloses her eggs, selected the scarab as a symbol of their god Khepera "he who turns" or "rolls." According to Horapollon and early Greek and Syrian writers, it was believed that no female of the species existed and that the male produced the egg, perpetuating his hypaxis by his own act, and thus the scarab became the emblem of the self-begetting and of the immortal, while in the hermetic literature it was looked upon as the type of the "only begotten," of "generation," of "father," of "man," of the "world." It has been stated that these latter ideas are for the most part foreign to the original Egyptian idea. It was supposed that Khepera caused the sun to move across the sky in the same manner as the scarab rolled its ball along the sand. The Egyptian name of the insect was kheper, from a stem meaning "to become, to come into being," and a picture of the beetle was the usual ideographic sign for the verbal stem and its derivatives. The deity Khepera typified the rising sun and was represented sometimes as a man with a scarab upon or in place of his head. It is said that the scarab was also the type of the human

soul emerging from the mummy just as the beetle was supposed to emerge and fly up toward the sun and heaven and thus the insect became a symbol of regeneration and of perennality. The daily appearance and reappearance of the sun represented the return of the soul to life.

Horapollo states that the number of the beetle's toes is thirty, five on each leg, symbolizing the days of the month, but Fabre has called attention to the fact that the number of tarsal joints is but twenty, the two front legs being devoid of tarsi. Horapollo writes, "The Scarab buries her ball in the ground where she remains hidden for twenty-eight days, a space of time equal to that of a revolution of the moon, during which period the offspring of the Scarab quickens. On the twenty-ninth day, which the insect knows to be that of the conjunction of the sun and moon and of the birth of the world, it opens the ball and throws it into the water. From this ball issue animals that are Scarabs." Fabre tells us that Horapollo was correct in his twenty-eight day statement, for approximately such a length of time is required for the larval stage, also that he was right concerning the necessary interposition of water to bring the adult beetle out. The ball is not thrown into the water, but rain softens the hard, baked shell of the ball and facilitates the emergence of its inhabitant.

Dow states, "the dentation of the fore tibiae of the scarab are thirty in number, the days of the celestial month, and those on the head resemble the sun's rays. Hence its dedication to Amen-Ra, the Egyptian God of the Sun Mystery." Mr. Andrew J. Mutchler, of the American Museum of Natural History, very kindly examined several specimens of *Scarabæus sacer* from different localities, including the Nile Valley, and advises me that in some specimens there are fifteen teeth on each tibia if one adds together the large and small ones and the terminal spur, but other specimens have more than fifteen on each tibia. The accompanying drawing furnished by Mr. Mutchler is of the right tibia of an example of *Scarabæus sacer* and shows fifteen such dentations.



The left tibia of the same beetle had one small tooth between the two large anterior ones, five between the two large middle ones and four between the last two large ones.



Right tibia of *Scarabæus sacer*.

According to Myer and other authorities, the scarab emblems of the Egyptians were cut from diverse materials into forms representative of a beetle and engraved around the circumference or on the base with hieroglyphic or other devices. The beetle form was either copied closely or conventionalized. One or all of the elytra, legs, prothorax might not be indicated and the insect might be cut high or low on its base. In addition to steatite being one of the earliest materials used, it is suggested that some were made probably from clay and then dried and cut into shape. Many were carved out of opaque or semi-transparent stones and those of hard stone were usually one of the following: green basalt, diorite, granite, haematite, lapis lazuli, jasper, serpentine, amethyst, agate, etc. A few were made of gold, ivory and wood. Following the cutting, they were glazed in fire with different colored enamels, usually of a light, bluish green. Petrie writes that "the earliest scarabs of Khufu are all small, of fine work, but without elaboration and of colours delicate and beautiful, and very permanent; even under Khafra, there is a deterioration in the glazing, which has often perished; and later on good glazes are rarer until a revival under the XIth dynasty." He says that the glazes of the XIth are "hard and unalterable and of fine colours; and some of those of the XIIth are very fine, but they have often decomposed. Blue is a special colour, as it is in the sculpture of that age." Also that all the brown scarabs, which are in the majority, were originally glazed with green, whereas all

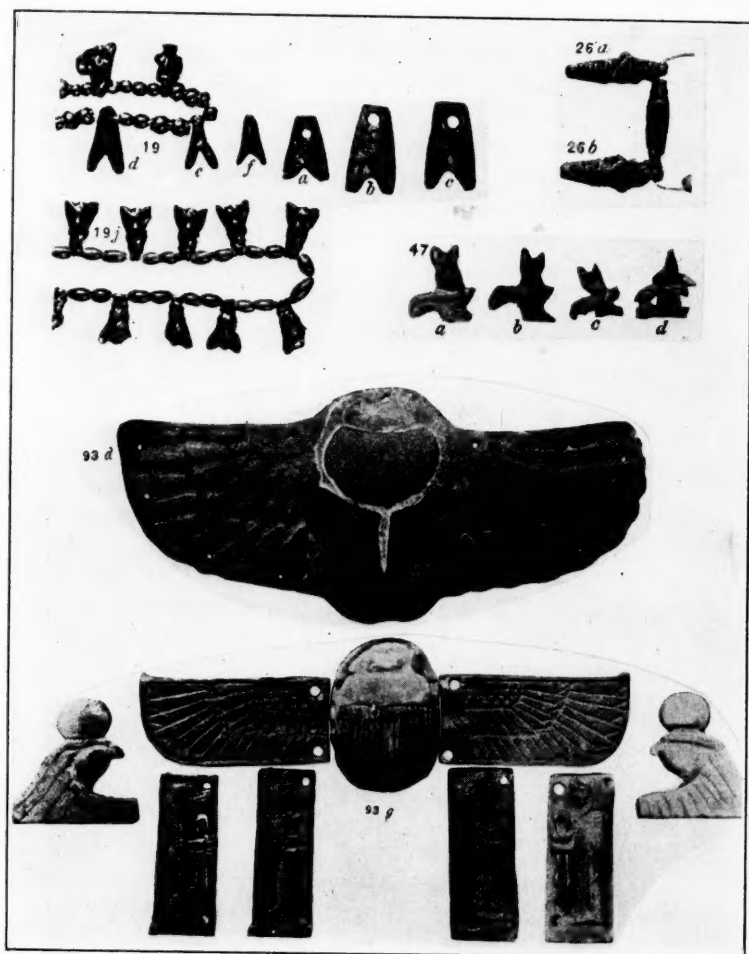


the white ones, except possibly some of Amenhotep III, were originally blue. There are also white and gray ones now devoid of glaze, which were originally either blue or green. He further states that the art of glazing greatly deteriorated after the XVIIIth dynasty and far the greatest part of succeeding scarabs have lost all traces of their original colors, being now mere browns and grays. "The work also becomes very poor under Ramessu II and his successors." "Some fine examples occur in the XXIVth and XXVth dynasties with a revival of both glaze and workmanship but a settled neatness and poverty overspread the XXVIth and is only broken occasionally." The workmanship varied in the different dynasties and this is thought to be due largely to changes in styles and in places of manufacture.

Myer classifies scarabs as follows: I, mythological or religious, bearing figures or inscriptions of a religious or mythological character; II, historical, with royal cartouches and devices relating to civil customs; III, physio-graphical, bearing animals and plants connected with sanctified symbols; IV, funereal, "connected with the Ka or life of the mummy in this world and with the journey of his Ba or responsible soul through the underworld"; V, talisman, or amulets to protect the wearer in this world from injury by evil spirits or men; VI, signets or seals for official and other uses; VII, others bearing designs, the meanings of which are in doubt.

According to the "Handbook of the Egyptian Collection of the Art Institute of Chicago," by Thomas George Allen, scarabs may be arranged as A, royal; B, official and private; C, divine; D, wish scarabs; E, decorative and symbolic; F, heart scarabs; G, miscellaneous. Petrie arranges scarabs into twenty-three classes based on details of workmanship, form and distribution, such classification being for the purpose of identification.

Allen states that "some scarabs with royal names were seals, but those bearing pre-Middle Kingdom names are pure amulets, none of them made earlier than the Empire, intended to invoke the aid of powerful deified sovereigns



## PLATE I

FIG. 19. Fly; 19a, green serpentine; 19b, c, pink limestone; 19d, red jasper; 19e, gold; 19j, string of yellow glaze.

FIG. 26. Locust; 26a, b, grass-green and yellow glazes.

FIG. 47. Hornet; 47a, yellow sard; 47b, orange sard; 47c, blue paste; 47d, blue glaze.

FIG. 93. Scarab Winged; 93d, blue paste with holes for stitching on a mummy network; 93g, green glaze with violet inlay in the hawk heads and four genii.

of the already hoary past." "Menes, founder of the I. Dynasty; Snefru, the III. Dynasty exploiter of mines in Sinai; the IV. Dynasty pyramid-builders Khafre and Menkure" are a few of the kings commemorated.

Official and private scarabs are largely of the Middle Kingdom-Hyksos date and as a group represent the seal use of scarabs. Some of the titles borne are, "the head storehouse-keeper, Hotpiy; the victualer of the ruler's table, Hori; the . . . of the beer-cellar, Pebes," etc. Newberry informs us that "the commonest form of Egyptian seal was that cut in the shape of a scarabæus beetle" and he divides the subjects engraved on scarab-seals into three groups—those bearing hieroglyphic inscriptions, those bearing figures of men, animals or flowers and those bearing geometrical designs, coil and rope patterns, etc. Such groups are then subdivided into other groups. In his book, "Scarabs, an Introduction to the Study of Egyptian Seals," Newberry treats scarabs mainly as seals, while admitting that a small number, compared with the seal class, were used as amulets and to commemorate historical events. Such seals range from one fifth of an inch in length to four or even five inches, the usual size being about three fourths of an inch long by one half inch broad and a quarter of an inch high. Newberry reviews the principal theories concerning the significance of Egyptian scarabs, such as the suppositions that they were used as tokens of value, as charms or amulets having magical qualities, as sacred emblems, and develops at length the theory that they were used mainly as seals or signets. He claims that such a use is borne out by the very large number of actual impressions of them found on bits of clay that have been used as seals to boxes, vases, bags, etc., found in ancient ruins, such impressions including every variety of scarab. The importance, origin and uses of the seal in ancient times are also explained fully. Newberry states:

It has been urged against this interpretation that the manufacture of scarabs in such profusion as we find them precludes the idea that they were

signets and nothing more, but it seems to have been forgotten that many millions of people must have lived during the several thousand years of ancient Egyptian history. The fact also that so many bear the royal superscription of one and the same king has likewise been brought forward as a serious objection to the theory that royal scarabs were used as seals; but here again the two kings whose names are most often found on these objects are the two—Thothmes III and Rameses II—whose reigns were the longest of all the Egyptian monarchs, and they must have employed a great number of officials entitled to use the royal seals during their long administrations.

Divine scarabs are those bearing names, titles or figures of various gods, which are of value as amulets. Concerning the so-called wish scarabs, which might be seals as well, the wishes are so often expressed in such a shortened way that the meanings are obscure. Some inscriptions read, "May Ptah give a Happy New Year from the Prince Shashanq to his mother Ka-ra-ma," "I am true of heart," etc. The decorative and symbolic scarabs include many varieties.

The heart scarabs are of considerable interest. It has been mentioned previously that Osiris dwelt in the lower regions as king of the dead. When the Egyptian died, he became as Osiris, "the nocturnal sun"; resurrected, he became Horus, the new-born and rising sun; in mid-day he was Ra. The birth and journey of man on earth was considered as similar to the solar journey. Wiedemann, in his "Religion of the Ancient Egyptians," writes that to the Egyptian the heart was the seat of life and according to the texts, resurrection of the dead who were without hearts was impossible. After death the heart was supposed to lead an independent existence travelling by itself through the under-world until it met the deceased in the Hall of Judgment. During the time between death and judgment, the Osiris was supposed to be without a heart and yet be alive and so the mummy was provided with a provisional heart for such a period, in the shape of an amulet of stone, either of heart-jar shape or in the shape of a large scarab, both of such objects being pledges of immortality. By virtue of this amulet the deceased was enabled to pass the ordeal of the weighing of the heart at the final judgment. The inscrip-

tion on such a scarab was usually Chapter XXX<sub>B</sub> of the "Book of the Dead." E. A. Wallis Budge in his work "Osiris and the Egyptian Resurrection" informs us as follows: "The speech which is put into the mouth of the deceased when in the Hall of Osiris during the weighing of his heart is the same in all the large illustrated copies of the Theban Recension of the Book of the Dead, and forms the section of that work which is commonly known as Chapter XXX<sub>B</sub>." This prayer is very old, and is said in the Rubric to Chapter XXX to date from the reign of Men-kau-Ra (Mycerinus). In the earliest times it was recited over a green stone scarab set in a silver-gold frame with a silver band over the back, but under the New Empire it was cut on the scarab itself. The scarab was placed inside the breast of the deceased and it was believed to "open his mouth for him, *i.e.*, give him back the power to eat, speak, think, remember, feel and walk, which he had enjoyed upon earth." Budge further states:

Thus we see that the beetle was associated with the idea of a renewal of vital power, and with new life generally. This idea is still extant in Africa, as we may see from the following extracts. The beetle referred to in the Rubric is the large beetle which is generally known as *Goliathus Atlas*, and many models of it in green stone are to be seen in the British Museum. A specimen caught by Dr. Junker, who figured it two thirds natural size in his book, was 10 cm long and 4 and one half cm wide. The wing-cases were brown, and on the black thorax there were broad white bands converging towards the head, while the sides of the abdomen and the legs were of a dark olive green color. Sir Harry Johnston says that the natives give much attention to the various species of the *Ceratorrhina goliath*, and speaks of it being much used in native medicine and sorcery. Mr. Torday also describes a magical ceremony in which the body of a goliath beetle plays a prominent part. Baker, in an entry dated August 11, speaks of "immense beetles" which appear at this season and make balls of dung as large as small apples, which they roll away with their hind legs, while they walk backwards by means of their fore-legs. They appear about the beginning of the wet season, and when the rains cease they disappear. As in one of his forms Osiris was a River-god it is extremely probable, as Baker suggests, that the Egyptians associated the appearance of these beetles with the rise in the river level, and therefore with new life and fertility. The beetles deposit their eggs in the usual manner, and while the larvae are growing they feed upon the ball of dung until they are ready to begin the world for themselves. That some tribes connect the beetle with ancestor-worship is proved by the

fact that Livingstone saw a large beetle hung up before a figure in a spirit-house of a burnt and deserted village. The modern Sudani women eat beetles and say that they make them prolific, and we may note in connection with the idea of new life which is associated with the beetle that the Egyptians used the shell of a beetle mixed with oil, etc., as a medicine to assist a woman in labor to give birth to her child.

In the Egyptian "Book of the Dead," Chapter XXX<sub>B</sub> is given as follows:

Title: A chapter about not letting the heart of the man act against him in the Netherworld.

Vignette: The deceased is in adoration before a scarabæus.

Line 1. My heart that I got from my mother, my heart necessary for my living on earth, do not rise against me, do not bear witness as an opponent to me among the circle of

2. gods on account of what I did before the gods; do not part with me before the Great God, the Lord of the Amenti.

3. Hail thou, O heart of Osiris, residing in the West; hail you, bowels; hail you, gods with a braided beard, august by your sceptre, speak well of the Osiris N, make him thrive by Nehebkau. I united myself with the earth

4. on the westernside of the sky. After lying on earth, I am not dead in the Amenti, I am there a pure spirit forever. *To be said upon a scarabæus of hard stone shaped and coated*

5. *with gold which shall be put into the man's bosom after his mouth has been opened and he has been anointed with head-oil. The following words are to be said upon him as a magic charm; My heart I got from my mother, my heart that is necessary to me for my transformations.*

Quoting Breasted:

Before Osiris, enthroned with forty-two assistant judges, hideous demons, each representing one of the nomes into which Egypt was divided, the deceased was led into the judgment-hall. Here he addressed his judges, and to each one of the forty-two assistants he pleaded not guilty to a certain sin, while his heart was weighed in the balances over against a feather, the symbol of truth, in order to test the truth of his plea. The forty-two sins, of which he says he was not guilty, are those which are condemned as well by the modern conscience of the world. They may be summed up as murder, stealing, especially robbing minors, lying, deceit, false witness and slander, reviling, eaves-dropping, sexual impurity, adultery, and trespass against the gods or the dead as in blasphemy or stealing of mortuary offerings. It will be seen that the ethical standard was high; moreover in this judgment the Egyptian introduced for the first time in the history of man the fully developed idea that the future destiny of the dead must be dependent entirely upon the ethical quality of the earthly life, the idea of future accountability.

If the heart came through the ordeal successfully, the dead received the title "true of speech" and passed on to

future happiness. Breasted states that the priests for their own profit sold heart scarabs inscribed with the charm Chapter XXX of the Book of the Dead and that when laid upon the breast of the mummy under the wrappings, the accusing voice of the heart was silenced in the presence of Osiris. In this manner Osiris and his forty-two assistants were outwitted by any impious person who had the means to buy a heart scarab.

Petrie believes that the Egyptians considered the beetle as sacred and that it was an emblem of the creator Khepera, this symbolism being a part of the aboriginal animal worship of Egypt. In view of this, he states that we have no right to separate it from the primeval idea that was connected with it in later times. He compares it with the Christian cross as a symbol and says that after the scarab had become commonplace with its everyday use, "it was resanctified in the XVIIIth dynasty by being carved in a very large size with a purely religious text upon it and placed in a frame upon the breast of the dead. On this frame it is often shown as adored by Isis and Nebhat. It is said to be the heart of Isis who was the mother of the dead person, thus identified with Horus." It is also stated by Petrie that in the twenty-third dynasty and later, the winged scarab was placed on the breast of the mummy "as the emblem of the Creator who should transform the dead, and associated always with the four sons of Horus as guardians of special parts of the body." From this time to the thirtieth dynasty many scarabs were placed on the mummy, "usually a row of half a dozen or more along with figures of the gods." Such scarabs were almost always carved with legs beneath and were devoid of inscriptions. Upon reaching gnostic times, there were three scarabs in a row as emblems of the Trinity, "with three hawks as souls of the just before them and three crocodiles, three snakes, etc., as souls of the wicked driven away, behind them." In this way the use of the scarab as an emblem of the Creator Khepera became in triple group the emblem of the Trinity.







Petrie further believes that the utilitarian view of the scarab is true in some cases but should not be held as the main purpose. In opposition to the theory held and elaborated by Newberry, concerning their use principally as seals, he states that scarabs were originally nearly all covered with a glaze, which has since disappeared, leaving the lines clear and that no impression could have been taken with the lines or most of them filled with the glaze.

Relative to their use in Egypt, according to Newberry, scarabs were not employed before the end of the sixth dynasty (2625-2475 B. C.) and then rarely. At the beginning of the twelfth dynasty their use was also much restricted, but at the middle of that dynasty, their use became general, being widespread by the time of Amenemhat III. From that time until the end of the twenty-sixth dynasty (663-525 B. C.) their history can be followed in a continuous line after which they became very scarce, finally disappearing early in Roman times. Their principal period of manufacture in large quantities, according to Myer, was during the reign of Thothmes III in the eighteenth dynasty (1580-1350 B. C.). Other times were the nineteenth and twentieth dynasties.

Concerning reissues of scarabs of an earlier king in a later reign, Petrie writes:

It is evident that we must always consider the age of a scarab independently of the inscription as it may be of any period subsequent to the king named. The great criterion showing that scarabs really belong to the age of the king they name, is in the fact that we find similar work and color in the majority of the scarabs of any one king, and each such style is different from that of any later age.

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#### PLATE II

FIG. 90. Inscribed Scarabs, backs; 90a, d, e, black steatite; 90b, brown limestone; 90e, glazed steatite; 90f, hard light brown limestone in silver mount; 90g, a metamorphic mud similar in material to slate; 90h, j, durite.

FIG. 90. Inscribed Scarabs; a, b, f, g, fronts of a, b, f, g, above.

FIG. 2. A plan showing the positions of amulets on a mummy.

The illustrations on the accompanying plates are from "Amulets" by Prof. W. M. Flinders Petrie, permission for their reproduction having been kindly given by the author.

Many scarabs were strung on threads and probably secured to the garment of the person wearing them. Others were mounted in metal rings or worn as bracelets or necklaces by the living. Small-sized scarabs, incised with hieroglyphics and perforated longitudinally, are found on the breasts of mummies, next to the skin or suspended from the neck by a wire or string, or mounted in a ring worn on the forefinger of the left hand or grasped inside the closed left hand. Heart scarabs were sometimes placed in the chest as a substitute for the heart which was removed. Sometimes they were located upon the eyes, or breast, or stomach, or strung into a net covering the corpse and sewed on the wrappings. As many as three thousand have been found in a single tomb. Sometimes a large winged scarab was made of successive layers of linen on the lids of outer coffin cases and painted in colors.

As to their geographical range, outside of the Nile Valley, where they have been found by thousands among the fragments of ruined towns and temples and in tombs on the edge of the desert, they have been discovered in all places that had commercial relations with Egypt, such as Greece, Syria, Aegean Islands, Crete, Italy, Sardinia and the north coast of Africa. In the "Catalogue of Engraved Gems in the British Museum," by Murray and Smith, it is stated, the scarab "found its way into Greece and Etruria partly through the commerce of the Phoenicians, and partly under the influence of Greek residents in Egypt during the sixth century B. C., or nearly so," and that "gems so peculiar in shape and in the designs engraved on them appear to have had no permanent attraction for the Greeks." The Etruscans, however, had a passion for gems of this kind and Etruscan scarabs copied from the Egyptians were changed in size, subject and ornamentation and were conventional and exaggerated likenesses of the insect. Scarabs with Egyptian, Chaldean, Assyrian and Persian subjects engraved on them and bearing inscriptions in Phoenician, Aramic or

Hebrew, have been found frequently in lands bordering the Mediterranean. These are supposed to have been manufactured mainly as articles of trade by the ancient merchants of Tyre and Sidon or were Egyptian or other originals inscribed by Phoenician lapidaries.

Ward affirms that scarabs as sacred emblems gradually became disregarded after the Persian period (525-332 B. C.) and practically ceased to be used as such. Scarab worship never gained support in Greece; in fact, the Greeks were disposed to make fun of it. Aristophanes, in his comedy "The Peace," given at Dionysia in 421 B. C., almost simultaneously with the end of the Peloponnesian war, alludes to it satirically, and has in the opening scene representing the outside of the house of Trygaeus, two servants attending to the wants of a gigantic dung-beetle, which is supposedly confined in an outer court, out of sight of the audience. However regardless of the theories concerning the significance of the scarab, all authorities are agreed as to the importance of these small objects of art in the study of the history and civilization of ancient Egypt.

In addition to the scarab, other insect models figured as amulets and were used by the Egyptians for the living as well as for the protection of the dead. Petrie informs us that the "great flood of amulets for the dead belong to the twenty-sixth to thirtieth dynasties" and that "many were used for the living in the Roman period." He divides them into five classes in accordance with their meanings: first, "those that act by similarity, such as parts of the body, in order to preserve those parts" and those that would ensure growth, protection from wild animals, etc.; second, those for conferring powers; third, amulets of property or models representing funeral offerings of food, furniture for use of the dead, etc.; fourth, protective amulets, and fifth, theosophic amulets of human-headed gods, animal-headed gods and animal gods.

In his book on "Amulets," the fly amulet is placed in

the first class, the collar of gold flies given to an active fighter in the eighteenth dynasty suggesting the fly as an emblem of activity or swiftness. Two forms exist, one with rounded wings and a sharper-bodied form with pointed wings. The periods for this amulet are prehistoric, twelfth and eighteenth dynasties and materials, yellow glaze, blue paste, gold, jasper, serpentine, glazed steatite, etc. Another insect model in this class is the locust, of periods prehistoric, XVIII and Roman, made from limestone, yellow glaze and green glaze and supposed to insure protection from locusts. In the second class is placed the hornet amulet, of the sixth to twelfth dynasties, carved from sard and carnelian, blue glaze, black limestone and brown agate, and meaning "royal power of Lower Egypt."

Among the amulets for protection appear the heart scarabs, with flat and inscribed bases, placed inside the body or on the chest; the scarab with legs for protection against quartan fever, evil eye and snake bite, placed on the throat, chest, stomach and left hand; the winged scarab meaning probably "protective power of the creator" and placed on the collar bone, breast and stomach. In the group of amulets of animal gods is found the green beetle, of the periods prehistoric to twelfth and made of green glaze, serpentine, carnelian and blackened limestone.

In addition to the meanings attached to the various kinds of amulets, the materials were supposed to possess important powers. Petrie lists the properties as recorded by Pliny and Bellucci, and a few of these follow. Sapphire, "for headaches; promotes contentment." Amethyst, "for intoxication; against spells, hail and locusts, and for access to kings." Garnet, "for widows, comfort in misfortune." Serpentine, "against headache and serpent bites." Amber, "for throat affections; on neck for fevers; against witchery."

## LITERATURE CITED

- Allen, Thomas George.  
    "Handbook of Egyptian Collection," Art. Inst., Chicago, 1923.
- Aristophanes.  
    "The Peace" (translated by B. B. Rogers), New York, 1924.
- Breasted, James Henry.  
    "A History of Egypt," New York, 1912.
- Brown, Brian.  
    "The Wisdom of the Egyptians," New York, 1923.
- Budge, E. A. Wallis.  
    "Osiris and the Egyptian Resurrection," New York, 1911.
- Cowan, Frank.  
    "History of Insects," Philadelphia, 1865.
- Davis, Charles H. S.  
    "The Egyptian Book of the Dead," New York, 1894.
- Dow, R. P.  
    "The Testimony of the Tombs," Bul. Brook. Ent. Soc., Vol. XI, pp. 25-33, 1916.
- Murray and Smith.  
    "A Catalogue of Engraved Gems in the British Museum," London, 1888.
- Myer, Isaac.  
    "Scarabs," Leipzig, 1894.
- Newberry, Percy E.  
    "Scarabs," London, 1908.
- Petrie, W. M. Flinders.  
    "Religious Life in Ancient Egypt," New York, 1924.  
    "Social Life in Ancient Egypt," New York, 1923.  
    "Amulets," London, 1914.  
    "Scarabs and Cylinders," London, 1917.  
    "Historical Scarabs," London, 1889.
- Wade, J. S.  
    "The Scarab: Emblem of Eternity," Canad. Ent., Vol. LIV, pp. 145-149, 1922.
- Ward, John.  
    "A Popular Treatise on Egyptian Scarabs in Art and History," London, 1902.
- Wiedemann, Alfred.  
    "Religion of the Ancient Egyptians," London, 1897.
- Wilson, Robert Forrest.  
    "The Living Pageant of the Nile," Indianapolis, 1924.

## MENDEL'S LETTERS TO CARL NÄGELI

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THE publication of Mendel's letters to Carl Nägeli by Correns in 1905 was a service to genetics which seems not to have been fully appreciated by most of those who have since written accounts of Mendel's life and work. These letters are masterpieces of clear thinking and cover a wide range of experimental work which would easily have formed the basis for a large monograph on hybridization. Had this been written and published with adequate illustrative material it seems certain that Mendel's laws would have been understood and their value recognized at least fifteen years before 1900. In what follows an attempt has been made to present in translation certain of the more interesting parts of these letters and to show the wealth of Mendel's unpublished data. Itis (1924) has discussed the letters at some length and has added some fragments of Nägeli's answers. He gives an excellent brief review of Nägeli's life, work, and theory of heredity and evolution, which partly explains why Nägeli failed to appreciate the value of Mendel's laws. Perhaps the chief reason was the fact that Nägeli did not believe that garden varieties have any importance in evolution. He also thought that plants are continually adapting themselves to their environment, so that he could not believe that Mendel's *Pisum* types would remain true. He had a deep distrust of amateurs and thought no one but a physiologist capable of dealing with the problems of heredity. It is a plain case of an irresistible piece of research meeting a mind made immovable by preconceived ideas and prejudice. Besides all this Nägeli was far more interested in the fact that he had found an ally in his *Hieracium* work than he was in

the *Pisum* results. The practical value of Mendel's laws was lost upon him and he felt that even though they were true for *Pisum* and generally applicable (which he seriously doubted) they had no bearing on evolution and adaptation. This viewpoint is still held by some scientists of repute to-day.

But apart from Nägeli's failure to appreciate Mendel's laws and the consequent discouragement which Mendel must have felt the letters contain much of interest. In the first letter (December, 1866) he states his reasons for writing to Nägeli.

The acknowledged value of your contributions to the definition and classification of wild-growing plant hybrids makes it a pleasant duty for me to submit this description of some investigations on artificial hybridization of plants to your friendly attention.

He comments on Gärtner's work as follows:

The results to which Gärtner arrived through his investigations are known to me; I have repeated his work and reexamined it in order where possible to see whether they agreed with the laws of development found for the plants which I have investigated. Try as I would, in spite of my trouble I could in no single case obtain a clear insight from it. It is very much to be regretted that this worthy man did not publish a detailed description of each of his investigations and a sufficient diagnosis for the different hybrid forms, especially for those which descend from like fertilization.

Entries like

Some individuals approached nearer to the maternal, others toward the paternal type or the progeny reverted more to the type of the maternal ancestor,

etc., are considered too general, too indefinite, so that one can not form a sure judgment. However, one can still, in most cases at least, learn this much, that the possibility of an agreement with *Pisum* is not excluded. Indeed a decision can only be expected from investigations in which the degree of the relationship between the hybrid forms and their ancestors are diagnostically based and not simply evaluated on the basis of the whole impression. Otherwise the letter is largely a statement of pro-

jected research on species—hybridization intended to test the *Pisum* results further. He speaks of himself as inexperienced and hindered by school duties and asks for cooperation. From marginal notes it appears that Nägeli answered, criticizing the formulae derived from the *Pisum* results as “only empirical and not rational” (!) and emphasizing the need for further proof that the segregants would remain constant after crossing.

Nägeli did not answer Mendel's first letter for two months. From a fragment of this letter given in Iltis (1924) I quote the following:

It appears to me on the whole that the investigations with *Pisum* are not concluded, but that they are only beginning. The mistake of all new experimenters is this, that they are far inferior to Kolreuter and Gärtner in persistence. I note with pleasure that you do not fall into this error and that you tread in the footsteps of both your distinguished predecessors. But you should surpass them, and in my opinion this is only possible and one can only advance hybrid theory if the research with one object be carried out exhaustively in all directions. Such a complete series of experiments which gives incontestable proofs for the weightiest conclusions we still lack altogether. If you have extra seeds of your hybrid fruits which you are not going to sow, I am quite willing to cultivate them in our garden in order to see how the constancy stands the test under other conditions. I also especially wish to obtain A, a (descendants of Aa) AB, ab, Ab, aB (descendants of AaBb). If you assent I must ask you to send the seeds to me very soon with new statements of their origin. I naturally leave the choice to your judgment and only note that I have neither much time nor very much room at my command.<sup>1</sup> I neglect to go into another point of your contribution since without the researches on which they are based I could only speak on supposition. Your intention to include still other plants in your research is excellent and I am convinced that with further different forms you will obtain essentially different results (with respect to the hereditary characteristics). It would be especially desirable if you can manage to carry out hybrid fertilization with *Hieracia*, as it ought in a short time to be the genus which will be best known with regard to intermediate forms.

Mendel took Nägeli's criticism in good spirit, saying:

<sup>1</sup> The *Pisum* work had been largely discontinued in 1863, so when Nägeli asked for seeds in 1867 Mendel feared that even the complex hybrid material which he still had might not germinate. Each packet was marked with dominant and recessive characters indicated and each contained seeds from a single plant. The expectations and their meaning were explained in detail. Iltis states that Nägeli grew them, but he apparently made no careful study of them, since he does not mention it.



I had expected that your honor would speak of my investigations with mistrustful caution; I would not do otherwise in a similar case.

But then taking up the points in detail he makes his case quite clearly. He discusses his work on species-hybrids which included an astonishing number of genera. He used *Hieracium*, *Geum*, *Cirsium*, *Aquilegia*, *Linaria*, *Calceolaria*, and *Tropaeolum*. He also described varietal crosses with *Zea Mays Major* (with dark red seeds)  $\times$  *Minor* (with yellow seeds) and one with *Zea Mays Major* (with red seeds)  $\times$  *Zea Cuzco* (with white seeds). He doubts the specific rank of the latter. For studies on color inheritance he mentions using varieties of *Ipomoea purpurea*, *Cheiranthus annuus*, and *Antirrhinum major*. The concluding paragraph is of interest:

The experiments necessarily proceed slowly. At first some patience is required, later things are bettered if several experiments are in progress at the same time. Then from spring to fall the interest is aroused daily and the trouble which the protégées require finds a rich reward. Should I be permitted to forward the solution of the problems through my research in any way, then I should consider myself doubly fortunate.

The third letter begins humorously.

My resolutions to study the *Hieracia* of this locality in their natural habitats during this summer are unfortunately to be only partially realized. Lack of time is chiefly to blame for it, but I also am no longer quite fit for botanical excursions since Heaven has afflicted me with overweight which makes itself felt on pedestrian trips especially in mountain climbing as a consequence of the general law of gravitation.

He describes the  $F_1$  species—hybrids of *Hieracium*, *Geum*, *Cirsium*, *Linaria* and *Verbascium*—in some detail and shows his eagerness about them.

I look forward to the coming summer with impatience since the progeny of several fertile hybrids will bloom for the first time. They should be very numerous and I only hope that they may repay the yearning with which I await them with much information concerning their life histories.

In the fourth letter he asked for seeds or living plants of certain *Hieracium* species, and Nägeli sent them.

He mentions his appointment as abbot in the fifth letter:

A great change has recently occurred in my circumstances quite unexpectedly, my unworthy self having been elected by the chapter of the monastery of which I am a member, as lifelong head. From my previous quite modest position as teacher of experimental physics I find myself suddenly placed in a sphere in which much is strange to me and it will require much time and trouble before I can feel at home in it. But that should not prevent me from continuing the beloved hybridization experiments. I even hope to be able to give them more time and attention until I am initiated into the work of my new position.

The last three letters are largely concerned with the progress of the *Hieracium* species-hybrid investigations. This work was undertaken in an attempt to understand the origin of intermediate types in the genus. Nägeli (1885) had found the genus difficult to classify because of the presence of intermediate types in nature. Mendel wished to discover whether they arise by hybridization and if so if they are of short duration due to the fact that they are more or less sterile or that they are more easily fertilized by pollen from their parents. He realized that it was possible that the presence of intermediates in the genus might be due to the origin of new variations of the Darwinian type. He was unable to obtain many successful hybrids among the *Archieracia* while from various hybrids of the *Pilosella* group his results were as follows.

- (1) The  $F_1$  may be of several types, even though the parents breed true.
- (2) The  $F_2$  may be like the  $F_1$  for several generations.
- (3) Two hybrids gave  $F_2$ 's containing a bewildering number of characters in different combinations.
- (4) Selfing tends to occur in spite of the presence of pollen of a different parent, but more seeds are obtained if plants are so treated.
- (5) Under unfavorable conditions pollen may not form in some species, whereas others produce an abundance of it.

Mendel clearly recognized in 1870 that his results with peas were directly opposed to those which he had obtained with *Hieracium*. He says:

Of the hybrids *H. praecaltum* (†) (*setigerum* N.) + *H. aurantiacum* and *H. praecaltum* (*Bauhini*) (†) + *H. aurantiacum* the second generation has bloomed and of *H. praecaltum* + *H. flagellare* the third. I can not on this occasion repress the remark that it is very impressive to observe that the

hybrids of *Hieracium* in comparison with those of *Pisum* present an exactly opposite state of affairs. We have here clearly to do with separate phenomena which are a part of a higher general law.

We now know that the *Hieracia* are often apogamous and that there are differences in chromosome number between species, two facts which adequately account for his difficulties with the genus. To have perceived that species and varietal hybridization are often different and to have clearly outlined the points in which they tend to differ is in itself a very valuable contribution to genetics.

In these last letters he also reports briefly that he has obtained results with *Matthiola*, *Zea* and *Mirabilis* which confirm the laws deduced from the *Pisum* investigations and says:

What Darwin in his "Variation in Animals and Plants" considered a strange account is to be taken as a model for the genera mentioned.<sup>2</sup>

He used *Matthiola annua* (hairy) vs. *glabra* (smooth) and flower color of *Mirabilis* and *Matthiola*. He found flower color work difficult because he lacked a color chart. In the eighth letter he states that he has worked for six years with flower color in *Matthiola*.

I had an assortment of thirty-six varied colors of *Matthiola annua* sent from Erfurt which still prove insufficient for my purpose.

No wonder he needed a color chart! He later reports:

The color investigations with *Matthiola* have made only slight progress in the present year in spite of the great number of plants investigated. From the results to date a conformity with *Pisum* is probable. The intensity of the color presents certain difficulties. Often instead of the expected color one appears which, if I may be permitted to say so, is of a higher or deeper color octave, or both appear at the same time and indeed not in a few but in a whole series of examples. On that account the sorting is very uncertain since one can easily separate plants which belong together or make the reverse error. One keeps numbers for the different color varieties which are useless for the deduction of a developmental formula. New plants will be taken for study this year; perhaps I will succeed in obtaining simpler series with those.

<sup>2</sup> And yet Nügel did not mention Mendel in his "Mechanisch-physiologische Theorie der Abstammungslehre"!

He also describes an experiment to test Darwin's and Naudin's hypothesis that one pollen grain is insufficient to fertilize an ovule and produce viable plants by placing a single pollen grain on each of eighteen stigmas of *Mirabilis Jalapa* and showed that one is enough. He planned to test the matter further to see whether two grains could function in fertilizing one ovule by placing two grains, one bearing a factor for yellow, the other for white flower color, on the stigmas of carnelian *Mirabilis* flowers. Since both  $F_1$  types were recognizable he would expect to recognize a combination effect if it occurred. The results of this experiment are not given, but it is clear that he expected to find the more vigorous pollen grain would fertilize the ovule. He states that carnelian and yellow  $F_2$  segregants show their characters as clearly as if they had never been combined and agrees with Darwin and Virchow's observations on the persistence of single characters in animals and plants.

He crossed two of the dioecious species of *Lychnis*—*diurna*  $\times$  *vespertina* and from three blossoms the seeds gave: 54 ♀ : 20 ♂, 43 ♀ : 15 ♂ and 54 ♀ :: 17 ♂ or approximately 3 ♀ 's : 1 ♂. He asks:

Is it simply chance that the male plants occur here in the 52:203 or 1:4 ratio or has this relationship the same meaning as in the first generation of hybrids with different parents? I am inclined to doubt the latter if only on account of the peculiar deductions which would be derived from this case. On the other hand one can not easily reject the question if one considers that the "Anlage" which function in development must be expressed in the organization of the primary cells from which the plant was derived, either simply those of the pistil or only those of the stamens and that this difference in the primary cells could possibly be due to the fact that the eggs as well as the pollen cells differed with respect to the sex "Anlage." However, I do not intend to drop the matter altogether.

The Franco-Prussian War interfered with the correspondence from 1870-1873 and then Mendel complains of increasing duties, plants left to the gardener and improperly cared for and labeled, and his eye trouble, which interferes with the delicate *Hieracium* hybridization work. The last letter summarizes his results to date.

He offers any or all of his material to Nägeli for further investigation. He does not say that this is to be the end of the correspondence and of active hybridization work, but one has the feeling that it is at least a preparation for such a contingency. Mendel ends this letter with a statement of his general conclusions concerning the importance of hybridization to evolution. He says:

It is well known that unfavorable changes in the vital conditions of men and animals may give rise to a reduced reproduction, a sexual weakening or even total sterility; whereby the ♂ part always suffers first (as in animals in captivity). It might be the same in plants. *H. Pilosella incanum* can not be well adapted to this climate. The air here appears to be too dry in summer for this plant, perhaps also too warm. In the year 1870, the May and June flowers were quite sterile, in the following year partially fertile, the single heads appearing towards fall were however fully fertile. Supposedly the cause of the sterility in the summer flowers lay in the bad condition of its own pollen since I was also unsuccessful in fertilizing *H. Auricula* with the same, while at the same time fertilization of the remaining *Pilosella* varieties was not difficult. Towards the end of August however a fertilization with the pollen of *H. Pilosella incanum* was successful. Gärtner was also convinced by his investigations that the masculine principal (as he expressed it) was always first affected. If that is really the case, then spontaneous hybridization in *Hieracium* should be due to a temporary disturbance, which, if it were repeated often or long continued, would finally result in the disappearance of the species involved, while one or the other fortunately organized hybrid descendant which was just suited to the existing telluric and cosmic conditions might succeed in taking up the struggle for existence with the unsuccessful and continue it through long periods until it also suffered a like fate.

It is obvious that Mendel was thinking of evolution as one interested in scientific truth and that he did not feel that this was inconsistent with his high position as abbot of Brunn.

Correns states that Nägeli wrote twice (in 1874 and again in 1875) after receiving Mendel's last letter (1873) but received no reply to either.

#### LITERATURE CITED

Correns, C.

1905. "Gregor Mendel's Briefe an Carl Nägeli, 1866-1873," Leipzig.

Gärtner, F. V.

1849. "Versuche und Beobachtungen über die Bastardzeugung im Pflanzenreich," Stuttgart.

Iltis, H.

1924. "Gregor Johann Mendel, Leben, Werk, und Wirkung," Berlin.

Mendel, G.

1866. "Versuch über Pflanzenhybriden Vern. d. Naturforsch. in Brünn," 4: 3-47.

1869. "Mitteilungen über einige aus künstlicher Befruchtung gewonnenen Hieraciumbastarde," *ibid.*, 8: 26-31.

Nägeli, C.

1884. "Mechanisch-physiologische Theorie der Abstammungslehre," München.

Nägeli, C. von, and Peters, A.

1885. "Die Hieracium Mitteleuropas," München.

SHORTER ARTICLES AND DISCUSSION  
ON THE WINTER FOOD OF THE TREE SWALLOW  
(*IRIDOPROCNE BICOLOR*) AND THE MYRTLE  
WARBLER (*DENDROICA CORONATA*)

BOTH the tree swallow and the myrtle warbler are known to delay their fall migration, often, in regions where the bayberry (*Myrica carolinensis*) abounds, and to feed upon the berries of this shrub until late in the winter. Often individuals of the latter species will winter over in such regions. The problem of food for such wintering-over individuals must be a severe one, and is answered, quite largely, by devouring the berries of *Myrica*, eked out, no doubt, by hibernating insects, spiders and the like, and eggs.

It was the writer's good fortune, while following a myrtle warbler through a scrubby patch, not far from New Brunswick, N. J., to chance upon a considerable flock of tree swallows sailing and veering over a field covered with a dense stand of bayberry. This was on December 19, of the year just passed, and the presence of these birds was noteworthy for the reason that the ground was covered with a light fall of snow, the temperature was about twelve above zero, and a sharp gale was blowing, so sharp, in fact, that the birds had difficulty in flying. The *Myrica* field, where these birds were seen, is a portion of a moorland kind of country, grassy and sparsely covered with scrub oaks and other low growth, and remnants of a once extensive woodland. The winds have full and unhampered sweep over this barren area, and a bleaker and less sheltered territory it would be difficult to find. From time to time the flock settled into the *Myrica* bushes to feed on the berries or to shelter from the wind.

On the following day (the 20th) the flock was watched, and forty-six individuals were counted. On this day the wind was blowing strongly from the north and the temperature was 16° F. The birds' only protection from the weather was that afforded by the bushes, a woodpile on the edge of the moor and the roof of a neighboring house, on the lee side of which the entire flock gathered, at intervals, in a closely compact mass (Fig. 1). These resting periods followed periods of feeding in the bushes, which were themselves preceded by periods of flying about. During the three succeeding days on which the writer watched these



FIG. 1. T, the flock of closely compacted Tree Swallows sheltered on the lee side of the roof. W. woodpile. M. *Myrica* bushes on the moor.

birds, this was the almost unvarying order of their activities: (1) resting on the roof, (2) flying about over the moor, (3) feeding in the bushes. Occasionally groups would rest in the bayberry bushes, close to the ground, or low down in the woodpile referred to, instead of returning to the roof. During the morning hours the birds collected on the eastern side of the roof or of the woodpile; and during the afternoon hours on the western side, except when a strong wind made the lee side the warmer, no matter where the sun was at that time. They roosted at night both on the roof and in the woodpile, and in the bayberry bushes.

Associated with the tree swallows were myrtle warblers, but their movements were erratic, and they would disappear for several days together and then appear again. They never, so far as seen, resorted to the roof to rest, but sheltered in the bushes, close to the ground.

The inhabitants of the house said that they had noticed the tree swallow flock (the like of which they had never seen before) and were of the opinion that their attention had first been arrested by it during the first week in December. They were asked to keep watch of the birds and reported that they gradually de-



creased in numbers after the first week in January and that none were seen after the twenty-fifth.<sup>1</sup>

The interest that attaches to all this is that connected with the question: how did these birds secure sufficient food to keep up their rapid metabolic processes, and this in the face of freezing temperatures and high winter winds, on an exposed moor? Moreover, here were a group of almost exclusively insectivorous birds living, apparently exclusively, upon waxy berries. The questions of the balance in the diet, and the total change in food habits led to an attempt at a solution.

Examination of the bushes showed that the birds had picked off entire berries, or had stripped the berries of their waxy secretion, and together with this the tiny nodules of the exocarp of the fruit imbedded in the wax. No other elements which could have furnished food was found on the bushes, and furthermore the birds (watched with 8X binoculars at short range) were never seen to eat aught save the berries. An examination of the excrement collected abundantly from under the eaves and the woodpile showed traces of no other substances than those derivable from the berries. The hard, stony endocarp ("stone") was ejected entirely, usually minus its coating of wax, and its coating of tiny exocarpal nodules. The birds could not have broken open the endocarp to get at the seed inside (it is indeed with difficulty that a person can crack open an endocarp with his molar teeth), hence the conclusion is that the birds were subsisting on wax and exocarpal nodules.

The waxy substance on the bayberry is not a true wax, but a fat, being chemically composed of glycerides of stearic, palmitic and myrtistic acids, with a small amount of oleic acid. Hence it is readily digestible. Tests made of the fat showed that it contained traces of protein and carbohydrates, probably rubbed off from the exocarpal nodules, since tests of these, finely comminuted, showed a rich content of both protein and carbohydrate. Here, then, in the fat and exocarp the birds were securing three important food elements, a fairly good avine diet from what seems to be, at first consideration, an unpromising source. And this diet, alone, apparently, was capable of sustaining the highly-pitched metabolism of these birds, for a month and a half, even

<sup>1</sup> Tree swallows as late as December 31st were reported by Chapman, at Englewood, N. J., in *The Auk*, Vol. 6, 1889, p. 199.

under such physiologically trying winter conditions as those described.

In the three days during which the writer had the birds under observation he never saw them leave the immediate vicinity of the bayberry patch and the house, nor take any other food than the bayberries.

It is suspected that the myrtle warblers likewise, at this season of the year, where they winter over in the bayberry thickets, subsist largely on the fat and exocarpal nodules of the berries.

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#### ON THE ABSENCE OF ISO-AGGLUTININS IN RATS<sup>1</sup>

MUCH interest has been shown in the question of blood groups by the medical profession since their discovery in 1900. Numerous investigators have confirmed their findings, and it has been established that at least four different blood groups are inherited in Mendelian fashion, apparently on the basis of two pairs of unit factors, although there are indications of a greater complexity.

More recently a number of investigators have been studying the question of blood grouping in animals. Here, however, the quest has been less successful, for no definite blood groups in animals have been demonstrated.

Among those reporting negative iso-agglutination tests are Hektoen (1907) on horses, cattle, rabbits and guinea-pigs; Weszeczky (1920) in guinea-pigs, rabbits, cattle and chickens; Rhodenburg (1920) in rats; MacDowell and Hubbard (1922) in mice; Panisset and Verge (1922) in horses and cattle; Przesmycki (1923) by iso-transfusion in rabbits; and Snyder (1924) in rabbits.

On the other hand, Ottenberg and Friedmann (1911) found that the blood of rabbits fell into four groups, although this disagrees with the findings of Snyder, Hektoen, Weszeczky and Przesmycki. The same authors report that the blood of steers fell into three groups. Ingebrigtsen (1912) found a few cases of inter-agglutination in cats, but he was unable to make a satisfactory grouping. Fishbein (1913) reported a similar inability to find groups in swine, cattle, sheep, rabbits, frogs and dogs, al-

<sup>1</sup> Paper No. 11 from the Department of Genetics, Iowa State College, Ames, Iowa.

though he found occasional cases of agglutination. A corresponding situation was reported in dogs by Ottenberg, Kalisky and Friedmann (1913), by Ottenberg and Thalhimer (1915) in cats and by Weszecky (1920) in a small number of trials on swine. In iso-agglutination tests on chickens and ducks Landsteiner and Miller (1924) report reactions of positive intensity in some instances, and in sheep Bialbsuknia and Kaczowski (1924) were able to classify the blood into three main groups and one subgroup.

While in general the blood cells of one species are agglutinated by the serum of another, Weszecky (1920) found that the serum of the buffalo did not agglutinate the cells of cattle. MacDowell and Hubbard (1922) reported that rat serum did not agglutinate mouse cells, and Walsh (1924) found that horse serum did not agglutinate ass cells, but that in most cases the ass serum did agglutinate horse cells.

In over three hundred trials the present writer failed to find any inter-agglutination of the cells of forty-six rats of five different strains, including Wistar strains A and B and the common wild rat of this locality (*Mus norvegicus*). Likewise, it was observed that rat serum failed to agglutinate mouse cells in a number of trials.

In carrying out these tests the rats were bled from the tail, under anesthesia, into sterile centrifuge tubes. After an hour or so the clot was broken, and the tubes set in the ice-box over night, after which the serum was pipetted off into sterile vials and preserved with 0.5 per cent. phenol. For carrying out these tests, blood was drawn into sterile centrifuge tubes containing 1 per cent. sodium citrate (one part of citrate to four parts blood) to prevent clotting. The macroscopic slide method was used for the tests, and the slides were read both macroscopically and with the low power of the microscope after standing for several minutes.

In general these results agree with the findings of other investigators who have studied iso-agglutination in animals. It seems quite certain, therefore, that blood groups do not exist, or at least rarely so, in most animals, thus making it impossible to study their inheritance in an experimental way.

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## LITERATURE CITED

- Bialbsuknia, W., and Kaczowski, B.  
1924. "On the Differentiation of Various Breeds of Sheep by Means of Serological Methods," *Jour. Immun.*, 9: 593-601.
- Fishbein, M.  
1913. "Iso-agglutination in Man and Lower Animals," *Jour. Inf. Dis.*, 12: 133-139.
- Hektoen, L.  
1907. "Iso-agglutination of Human Corpuscles," *Jour. Inf. Dis.*, 4: 297-303.
- Ingebrigtsen, R.  
1912. "The Influence of Iso-agglutinins on the Final Results of the Homoplastic Transplantation of Arteries," *Jour. Exp. Med.*, 16: 169-177.
- Landsteiner, K., and Miller, C. P., Jr.  
1924. "On Individual Differences of the Blood of Chickens and Ducks," *Proc. Soc. Exp. Biol. and Med.*, 32: 100-102.
- MaeDowell, E. C., and Hubbard, J. E.  
1922. "On the Absence of Blood Groups in Mice," *Proc. Soc. Exp. Biol. and Med.*, 20: 93-95.
- Ottenberg, R., and Friedmann, S. S.  
1911. "The Occurrence of Grouped Iso-agglutination in the Lower Animals," *Jour. Exp. Med.*, 13: 531-535.
- Ottenberg, R., Kaliski, D. J., and Friedmann, S. S.  
1913. "Experimental Agglutinative and Hemolytic Transfusions," *Jour. Med. Res.*, 28: 141-163.
- Panisset, L., and Verge, J.  
1922. "Sur l'existence des groupes sanguins chez les animaux," *Compt. Rend. Soc. de Biol.*, 87: 870-872.
- Przesmycki, F.  
1923. "Researches on Transfusion of Blood in Horses and Rabbits," *Exper. and Social Med. (Warsaw)*, 1: 2-3.
- Rhodenburg, G. L.  
1920. "The Iso-agglutinins and Iso-hemolysins of the Rat," *Proc. Soc. Exp. Biol. and Med.*, 17: 82.
- Snyder, L. H.  
1924. "Iso-haemagglutinins in Rabbits," *Jour. Immun.*, 9: 45-48.
- Snyder, L. H.  
1924. "The Inheritance of the Blood Groups," *Genetics*, 9: 465-478.
- Walsh, L. S. N.  
1924. "The Blood Inter-relationships of Horses, Asses and Mules," *Jour. Immun.*, 9: 49-55.
- Walsh, L. S. N.  
1924. "Hemagglutination in Horses," *Jour. Immun.*, 9: 57-73.
- Weszeckzy, O.  
1920. "Untersuchungen über die gruppenweise Hämagglutination beim Menschen," *Biochem. Zeitschr.*, 107: 159-171.

